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ERRATA AND AUTHORS' EMENDATIONS

Page 67, first figure in column 3 of Table 1, "0.051" should be "0.51."

Page 286, fifth line from bottom, "usages" should be "usage."

Page 287, line 3, delete the words "present usage."

Page 316, line 7 in first paragraph under "Experimental Results," "seed produced," should be "produced seed."

Page 318, fifth and sixth lines from bottom should be "Cowpeas. *Vigna catjang sinensis:* Early Buff. Whippoorwill from Georgia."

Page 408, line 12 in second paragraph under "Emergence in Screen Cages," "Table 2, A–G, and Figure 7, A–G" should be "Table 2, A–E, and Figure 7, A–E."

Page 503, in list of plants, delete the word "Chinese" under heading "Common name" opposite ***Pyrus amygdaliformis*, *P. balansae*, *P. canescens*, *P. colinifolia*, *P. paschia***, and ***P. sinaica***.

Page 503, in footnote *a* the word "oriental" should be "foreign."

Page 514, legend for Figure 7, "1 with Hiley" should be "2 with Hiley."

Page 600, line 8 in second paragraph, "Willis and Carrero (*15*)" should be "Gile and Carrero (*2*)." In following paragraph, line 12, "Willis and Carrero" should be "Gile and Carrero."

Page 617, Figure 9, B is upside down, and the letters placed on the wrong tubers. Transpose "*a*" and "*b*."

Page 636, Figure 3. Neither the actual nor the theoretical curve should go as high as shown. The actual curve should be broader and flatter at the apex, reaching its peak at ordinate 165.5 and abscissa 263, then passing to abscissa 264 at ordinate 180.5, then to abscissa 263 at ordinate 185.5 from which point it descends as shown. The theoretical curve should go only as high as abscissa 270.

Page 637, the formula just above Figure 4 should be a part of footnote 3 on page 636.

Page 645, the sentence beginning in line 11 should read: "Considering the error of three replications 1/40-acre plots as 2.837 per cent, the expected error, instead of 1.959 which was actually found (Table 5), three times the difference between two replicated series is 12 per cent."

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INHERITANCE OF RESISTANCE IN OATS TO PUCCINIA GRAMINIS AVENAE[1]

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INTRODUCTION

The production of disease-resistant plants by hybridization has engaged the attention of biologists with increasing interest since 1878, when Darwin (*6*)[3] reported the production by James Torbitt of a fungus-proof potato. It was not until 20 years later, however, that Farrer (*10*) demonstrated the production of rust-resistant cereals. Varietal resistance, hybridization for the production of new resistant varieties, and the factorial analysis of the inheritance of resistance have been extensively studied during the last decade.

Henning (*15*), in Sweden, and later, Hungerford and Owens (*16*), in this country, have shown that there is a marked difference in the susceptibility of wheat varieties to *Puccinia glumarum* (Schm.) Erikss. and Henn. Hungerford and Owens, in greenhouse and field tests, showed many of the common wheats to be resistant. In 1920 Melchers and Parker (*20*) reported three Crimean hard winter wheats that were resistant to leaf rust, *P. triticina* Erikss.

Mains and Leighty (*18*), working with rye, an open-pollinated plant, found that 68 different selections were resistant to *Puccinia dispersa* Erikss.

The reaction of oat varieties to *Puccinia coronata* Corda and *P. graminis* Pers. has been studied by Parker (*23*). Of the 120 strains tested, 80 were susceptible to both rusts. White Tartar and Ruakura Rustproof proved resistant to *P. graminis*, while Burt and several others of the red-oat group (*Avena byzantina* C. Koch) were resistant to *P. coronata*. In 1920 Durrell and Parker (*8*) made a comprehensive survey, involving the assembling of data for five years, on the response of oat varieties to crown and stem rusts under field conditions. White Russian and Green Russian were found to possess a marked resistance to *P. graminis*. In a previous report (*7*) by the present writer, Richland (Iowa No. 105) was shown to be resistant to stem rust.

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[1] Received for publication Apr. 17, 1928; issued September, 1928. The investigations here recorded were conducted by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station. This article and the one entitled "The Alternate Hosts of Crown Rust, *Puccinia coronata* Corda," in the Journal of Agricultural Research 33: 953-970, 1926, were submitted by the writer to the graduate faculty of the Iowa State College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his thanks to I. E. Melhus, plant pathologist of the Iowa Agricultural Experiment Station, and to C. R. Ball and H. B. Humphrey, of the Office of Cereal Crops and Diseases, for suggestions and criticisms during the progress of the work and the preparation of the manuscript; also to the many assistants who ably aided in the collection of the data presented.

[3] Reference is made by number (italic) to "Literature cited," p. 22.

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Although the varietal response to the rusts is known, it often is necessary to produce new varieties by hybridization. Biffen (*3, 4, 5*) found susceptibility to *Puccinia glumarum* dominant and secured a monohybrid segregation in the F_2 generation. On the other hand, Nilsson-Ehle (*22*) concluded that distinct dominance of susceptibility to this rust was of rare occurrence. Hayes, Parker, and Kurtzweil (*14*), in their investigation of varietal resistance of wheat to *P. graminis tritici* Erikss. and Henn., found the F_2 and F_3 generations segregating for resistance and susceptibility, although sterility probably prevented an expression of the true ratios. Parker (*24*) showed that resistance to *P. coronata* was heritable and suggested a multiple factor explanation. In 1922, Garber (*11*) found resistance in oats to stem rust, *P. graminis avenae* Erikss. and Henn., to be dominant and due to a single-factor difference in Minota-White Russian and Victory-White Russian crosses.

Stakman and Levine (*26*), Melchers and Parker (*19*), and others have shown the existence of 37 physiologic forms of rust within *Puccinia graminis tritici*, and Stakman, Levine, and Bailey (*27*) later isolated four such forms within *P. graminis avenae*. Because these specialized forms exist, it is imperative to determine the response of wheat and oat varieties to each of them. Puttick (*25*), Aamodt (*1*), Harrington and Aamodt (*12*), and Hayes ,and Aamodt (*13*) have studied the reaction of wheat hybrids to many of these forms.

The possible existence of linkage between rust resistance and other characters has been shown by Hayes, Parker, and Kurtzweil (*14*) and by Waldron (*28*) in wheat, and by Garber (*11*) in oats. In none of these cases was the linkage sufficiently close, however, to prevent the production of varieties which were rust resistant and endowed with other desirable characters.

The manner of inheritance of rust resistance in wheat has been demonstrated by Hayes, Parker, and Kurtzweil (*14*) to be different for a durum-common and an emmer-common cross. Whether such a difference can be obtained in oat species and varieties has not previously been known. It is the purpose of this paper to report the manner of inheritance of resistance to *Puccinia graminis avenae* by eight varieties of oats belonging either to *Avena sativa* L. or *A. byzantina* C. Koch.

METHODS AND MATERIALS

Preceding a consideration of the inheritance of rust resistance in oats, adaptations of existing methods and the development of many new ones were necessary. The fact that many data were taken on each individual plant necessitated detailed planting, harvesting, and recording methods, together with special means of producing an epidemic of the rust and estimating its subsequent effect on these plants.

SOURCE OF OAT VARIETIES

The eight pure-line varieties of oats used as parent material of the hybrids treated in this study are described below.

The strain of Burt was a head selection from C. I. No. 710 [4] in 1916. It is a reddish black oat belonging to the species *Avena byzantina* and is susceptible to *Puccinia graminis avenae* but moderately resistant to *P. coronata*.

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[4] Cereal Investigations accession number.

The Early Ripe variety was a head selection from the material obtained by J. H. Parker, formerly of the United States Department of Agriculture, from H. H. Love, of Cornell University, Ithaca, N. Y. It is similar to Burt except that it has a finer straw and usually a lighter colored grain. It has fewer basal hairs and a less pronounced cavity at the base of the first floret. It is susceptible to both oat rusts.

The Green Russian variety was a selection from Minnesota No. 350 and belongs to the species *Avena sativa.* It is resistant to stem rust and moderately resistant to crown rust.

The Richland (Iowa No. 105) variety is a yellow selection from Kherson produced by L. C. Burnett, of the Iowa Agricultural Experiment Station, in cooperation with the United States Department of Agriculture. The material used here was a head selection from this pure line. It is an extremely early oat, an excellent yielder, and resistant to *Puccinia graminis avenae.* It often escapes *P. coronata* under field conditions in Iowa because of its early maturity.

The Lincoln variety was obtained from H. H. Love, of Cornell University. It is similar to Swedish Select, except that two-kerneled spikelets predominate. It yields well in the cooler sections of the United States, especially New York, but is susceptible to stem rust.

National, a variety similar to Silvermine, also was obtained from Cornell University. It is a good yielder, of mid-season maturity, but susceptible both to *Puccinia graminis avenae* and *P. coronata.*

The Ruakura variety was obtained from a head selection made in 1916 from C. I. No. 701. It is a slender-stemmed, early-maturing oat belonging to the species *Avena byzantina.* Ruakura is similar to Burt in vegetative growth, but has pubescent nodes. It possesses a moderate degree of resistance to both oat rusts.

According to Etheridge (*9*), the White Russian and White Tartar varieties are indistinguishable. White Russian was a head selection from Minnesota No. 5, whereas White Tartar was obtained from Cornell University. Both of these are side-panicled, late-maturing, low-yielding strains, belonging to *Avena sativa* var. *orientalis.* They possess marked resistance to *Puccinia graminis avenae.*

SOWING

The seeds were sown in 10-foot rows spaced 1 foot apart. In 1920, the individual seeds were spaced 6 inches apart in the row, but this distance was reduced to 4 inches in all the subsequent years of the experiment. In both the F_1 and F_2 sowings, only the primary kernel was used, the second floret or "pin" oat having been removed. This method permitted only one plant to emerge in a single space, thereby removing the difficulty of separating heavily tillered plants.

The F_1 seeds were divided into three lots. The first portion was sown in 1920 and produced the F_2 generation. F_2 plants were grown from the second portion in 1921 adjacent to the F_3 generation and compared with it. The third portion, with a similar remnant of F_2 seed and some of the original crossed seed, was sown in 1922, so that the F_1, F_2, and F_3 generations were grown side by side in that year.

Two series of control rows were necessary. Every tenth row was sown to the same susceptible pure-line variety to gauge the uniformity of the epidemic produced in the nursery, and the resistant or susceptible pure-line parents were sown every twenty-fourth and twenty-fifth row, respectively, in order to compare their rust response with that of their progeny. The culture of the nursery was uniform.

RECORDING

Each cross was given a number. For instance, White Russian×Burt was given number 274, which was used for all subsequent generations. Each F_1 plant of this cross or its reciprocal was given a subnumber; that is, 274–1, 274–2, 274–3, etc. Reciprocal crosses were made and their progeny studied to determine the differential influence, if any, on the progeny. No differences were noted, however, and in order to simplify the presentation of the data a cross and its reciprocal are considered as a single cross.

Each plant was given a position number. This method allowed for comparison of the response of each individual plant with each adjoining plant in the office records as well as under field conditions. The following data were taken on each plant: Dates of seeding, heading, maturing, and harvesting; percentage of rust infection; size of uredinia; height; shape of panicle; and yield.

HARVESTING

All of the individuals of the F_1 and F_2 generations were harvested separately. The panicles of each plant were wrapped in a separate paper and the product of the entire row was inserted in a paper bag. Each F_3 plant was labeled with a string tag, and the plants in the entire row were bagged together but threshed as individual plants.

TECHNIC OF PRODUCING EPIDEMICS

The classification of the individual plants into either the resistant or the susceptible group depended upon their relative response to inoculation by *Puccinia graminis avenae*. Theoretically, this classification was based upon the fact that all plants had equal opportunity for maximum infection. In order to afford this opportunity, the following methods of exposing the plants were used:

(1) Oat plants infected with stem rust were transplanted directly into the nursery from the greenhouse. These plants had been infected with urediniospores collected from the nursery in the previous year and maintained in greenhouse stock cultures.

(2) Urediniospores were scraped from oat plants in the greenhouse, placed in distilled water, and sprayed on the culms with an atomizer.

(3) Urediniospores were scraped from oat plants in the greenhouse and dusted on the previously moistened oat plants in the field.

(4) Urediniospores were applied directly to the leaves of the oat plants by means of a scalpel between 7 and 8 o'clock p. m. The plants thus exposed were covered with a bell jar for 12 hours.

In 1919 and 1920 plants in the field were exposed to infection when 6 to 9 inches high. It was noted, however, that very few became visibly infected until the panicles had just emerged from the sheath. From 1921 to 1923, therefore, the plants were not exposed until this stage of maturity was reached.

CLASSIFYING PLANTS BY SIZE OF UREDINIA

Each individual plant was classed as resistant or susceptible by using the scale devised by N. A. Cobb in Australia and later revised and used by the Office of Cereal Crops and Diseases, United States

Department of Agriculture.[5] This scale is based on the proportion of the total area covered by uredinia, no consideration being given to the relative size of the sori, though it will be shown later that the size of the rust sorus can be correlated directly with resistance. In addition to the quantitative estimate of rust, the size of uredinia is used in this paper in classifying the response of the individual plants to rust.

Rust estimates were started on the standing plants about three weeks before harvest and continued until harvest. During the last week of this period the plants were harvested and estimated at the same time. Only one rust estimate was made for each plant. The whole plant was inspected and the maximum infection accepted as indicating its degree of resistance. It was not uncommon to find certain culms of a single plant more heavily infected than others. Uredinia and telia were estimated collectively during the last few days of each season, for the fungus rapidly enters the telial stage as the oat plant matures. Progenies of all plants which could not be classified as either susceptible or resistant under field conditions were tested in the greenhouse or in the field the following spring.

As noted above, the size of the uredinia was employed as a measure of susceptibility. (Fig. 1.) During the progress of this work the resistant pure-line varieties of oats developed a consistently low percentage of infection. Many of the susceptible varieties varied in reaction from apparent resistance to susceptibility. These results could be explained in one of two ways: (1) The pure-line varieties of oats were not homozygous for susceptibility to rust; or (2) they were homozygous and certain plants were escaping infection or at least severe infection. It then became necessary to find some other index by means of which these escaping plants might be classified according to their inherent response to rust. Such an index was found in the size of uredinia.

If, then, the size of uredinia can form the basis for differentiating susceptible and resistant plants, how are small and large uredinia to be distinguished? Although these two classes were only relative, they none the less were sharply defined. Hence the classification was reasonably accurate because the greater dimension of the large uredinia was more than ten times that of those classified as small.

Pure-line resistant varieties of oats, under field conditions, consistently showed a low percentage of infection and small-sized uredinia. Pure-line susceptible varieties differed greatly in percentage of infection, but uniformly produced large uredinia. In progeny tests of 100 individuals, having a low percentage of infection but large uredinia under field conditions, 1,649 plants were produced the next year with a high percentage of infection and large uredinia. Clearly, then, these plants had partially escaped stem rust the first year but could have been classified as susceptible on the basis of size of uredinia.

Although pure lines showed a positive correlation between size of uredinia and response of the host to rust, it was not known whether hybrids acted in the same way. In addition to the data on size of uredinia on F_1 plants recorded in Table 1, records of F_2 and F_3 plants of Green Russian × Richland and White Russian × Burt, and F_4

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[5] Scale for estimating rust. In Cereal disease field notebook. U. S. Dept. Agr., Bur. Plant Indus., Cereal Invest., C. I. form 11. [June, 1915.]

plants of White Tartar × National and White Tartar × Lincoln were made. The susceptible F_1 plants, subsequently classified as susceptible by the reaction of both the F_2 and F_3 progeny, had large uredinia. An F_3 progeny test includes the F_3 individuals from a single F_2 plant. The F_1 individuals classified in Table 1 as resistant, and subsequently proving to be so, had small uredinia. In the total F_2 population in all years, 10,004 plants were classified as resistant and had small uredinia, while 713 were susceptible, with large uredinia. In the test of the F_3 progeny, those rows segregating for rust reaction also segregated for size of uredinia, the individual susceptible plants having large uredinia.

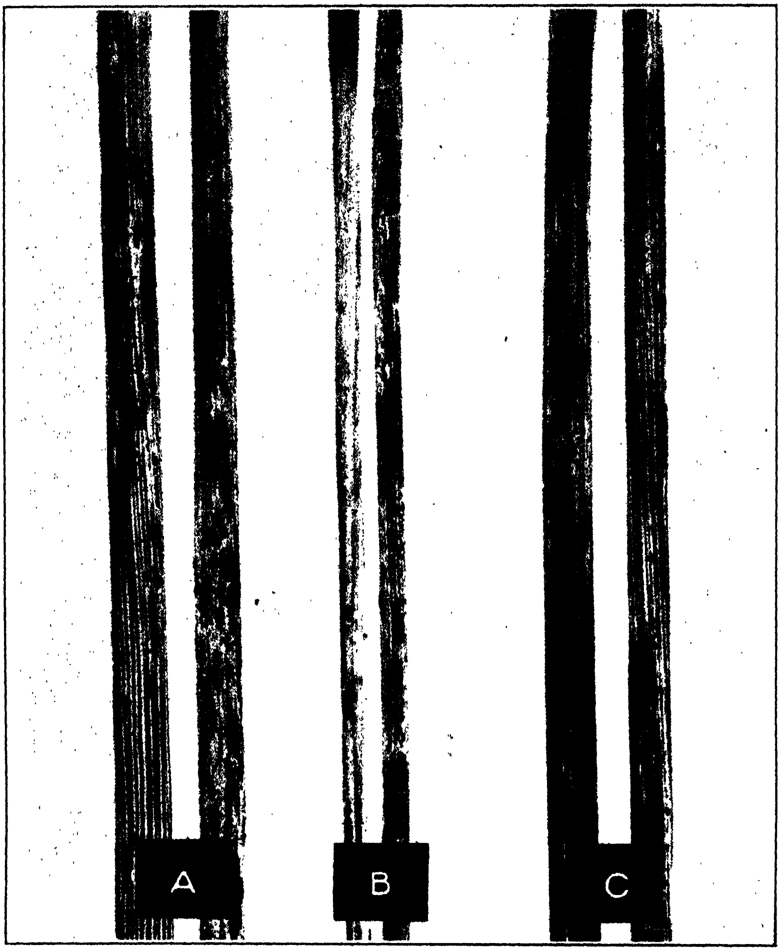

FIG. 1.—Size of urediniosori in an F_3 progeny of White Tartar×Lincoln: A, Large urediniosori (susceptible plant); B, intermediate urediniosori (susceptible plant); and C, small urediniosori (resistant)

Among the 2,700 F_2 plants studied for size of uredinia in 1920, all those placed in the large-uredinia group showed susceptibility in further progeny tests.

TABLE 1.—*Reaction of F_1 plants to Puccinia graminis avenae, showing correlation between size of uredinia and rust susceptibility*

Cross	Hybrid No.	Number of seeds set	F_1 reaction to P. graminis avenae (number of plants)			
			Resistant		Susceptible	
			Size of uredinia		Size of uredinia	
			Large	Small	Large	Small
White Russian×Burt	274	27	0	15	12	0
Green Russian×Burt	283	10	0	2	8	0
Siberian×Burt	1206	6	0	0	6	0
White Russian×Ruakura	271	2	0	0	2	0
Green Russian×Early Ripe	280	2	0	2	0	0
Green Russian×Richland	277	5	0	5	0	0
Total		52	0	24	28	0

It should be pointed out that not all plants having small uredinia are resistant, but that any of these plants, grown under field conditions, could be classed as susceptible if large uredinia were present on the culms, regardless of the percentage of infection.

Time of maturity perhaps is one of the chief causes for lack of perfect correlation between size of uredinia and resistance. All of the 100 rust-escaping plants mentioned above were mature from 7 to 10 days earlier than their susceptible sisters. It is probable, then, that maturity, accompanied by senility of the host cells, prevented further development of this obligate parasite. This conclusion is supported by the fact that many of the susceptible early-maturing plants had both large and small uredinia, indicating that the uredinia develop but little after the maturity of the host. Progeny from these plants, when infected at an earlier stage in the next year, were susceptible and produced only large uredinia.

The correlation of size of uredinia and rust reaction under greenhouse conditions is not so marked as in the field. As all stem-rust inoculations were made on the leaf blades in the greenhouse and on the culms in the field, some difference would be expected. Type of infection indicated by hypersensitive areas assisted in differentiating the oat plants grown in the greenhouse into either resistant or susceptible groups.

HYBRIDIZING IN THE GENUS AVENA

Before the production of oat hybrids was begun in 1919, a survey of the literature was made, but this revealed little information on the technic of crossing oat varieties under field conditions. Although field crossing can be done very successfully in some sections of the United States, in other sections successful crosses are obtained with difficulty owing probably to such factors as temperature and humidity. In the opinion of the writer, field crossing should be practiced only when greenhouse facilities are not available, as oats sown in the greenhouse in November, and heading in March, permit a high percentage of successful crosses. A consideration of such problems as length of time between emasculation and pollination, time of day best suited for pollinating, and quantity of pollen necessarily precedes the successful production of oat hybrids.

PERIOD BETWEEN EMASCULATION AND POLLINATION

In making an oat cross, the anthers must be removed from the inclosing lemma and palea before the pollen is shed on the stigmatic surface. It would be more convenient to remove the anthers and immediately insert the foreign pollen. Using White Russian as the female parent and Burt as the male, pollinations were made at the time of emasculation and at the end of each succeeding 12-hour interval up to 72 hours under field conditions. The first series, consisting of 10 pollinations on each of two panicles, was made at 7.30 o'clock a. m., the next at 7.30 o'clock p. m. The general method of pollination for all hybrids reported below involved cutting all except 10 spikelets from the panicle of the female parent on emergence from the sheath. The second floret was removed from each of these spikelets and the remaining primary florets were emasculated. An oil-paper bag was then placed over each prepared panicle and tied at the base with a string.

The results showed about the same percentage of seeds produced from pollinations at each of these periods. However, in testing these seeds, the F_1 plants indicated that 6 out of 20 of the resulting seeds were self-fertilized when emasculation and pollination occurred at the same time. A higher percentage of hybrids was produced when pollination occurred 48 hours after emasculation.

EFFECT OF TIME OF DAY ON SEED SETTING

Using the White Russian × Burt cross, the influence of time of day on set of seed was determined under field conditions. Pollinations were made at Ames, Iowa, and Iron River, Wis., at intervals from 4.30 o'clock a. m. until 8.30 o'clock p. m. on three consecutive days at each place.

At these two places 770 pollinations were made on 77 different panicles. The results are presented in Table 2. From these pollinations 78 seeds resulted, an average of 10.1 per cent for the entire experiment. From the forenoon pollinations 36 seeds were obtained and 42 from those made in the afternoon. During the six days no seed was set from pollinations made between 11.30 a. m. and 2.30 p. m. The failure to set seed on June 28 at Ames is difficult to explain, as the mean temperature and general characteristics were similar on these three days except for low relative humidity on June 28.

At Ames, out of a total of 180 pollinations made in the forenoon 17 were successful, as compared with 9 from a total of 210 pollinations made in the afternoon. At Ames no seed was set from pollinations made between 10.30 a. m. and 3.30 p. m.

At Iron River, Wis., 19 out of a total of 180 attempts were successful in the morning and 33 from a total of 210 in the afternoon. The interval during which no seeds were set was about two hours shorter at Iron River than at Ames. It is probable that such factors as temperature and relative humidity influence the effectiveness of pollination at some hours of the day.

TABLE 2.—*Influence of time of day on effectiveness of cross-pollinating White Russian and Burt oat varieties under field conditions*

Place and date in 1919	Number of seeds resulting from cross-pollination at time of day indicated												
	Forenoon						Afternoon						
	4.30	5.30	8.30	9.30	10.30	11.30	12.30	2.30	3.30	4.30	5.30	7.30	8.30
Ames, Iowa:													
June 26	4	2	1	2	0	0	0	0	0	0	1	1	----
June 27	2	2	4	0	0	0	0	0	0	2	1	3	1
June 28	0	0	0	0	0	0	0	0	0	0	0	0	0
Iron River, Wis.:													
July 29	2	3	1	0	0	0	0	0	0	1	1	1	2
July 30	1	1	1	0	0	0	0	0	0	3	2	2	3
July 31	2	1	1	3	3	0	0	0	4	3	4	3	4
Total	11	9	8	5	3	0	0	0	4	9	9	10	10

QUANTITY OF POLLEN

Although Jelinek (*17*) draws no conclusions concerning the quantity of pollen necessary to fecundation, this may be a factor in the explanation of his results in the production of wheat hybrids by the following methods: (1) Insertion of a whole anther in the female floret, and (2) emasculation of spikes and bagging them together with spikes of similar maturity belonging to the pollen parent. He states that in 1916 the second method resulted in twice as much seed production as the first. In the unfavorable year of 1917 no seed was produced by the first method, whereas 50 per cent of the florets on 24 out of 47 spikes produced seed by the second method.

An easy method of pollination is to insert a whole anther from the male parent between the palea and lemma of a floret of the female parent. However, when using this method in studies here recorded, fewer seeds developed than when a small quantity of pollen was dusted on the stigmatic surface, even though all pollinations were made early in the morning and at least 24 hours after emasculation. Upon examining the florets of crosses made by inserting the whole anther between the lemma and palea, many molds were found growing abundantly on the surface of the caryopsis. The excess pollen probably served as a favorable medium for these molds and their action often prevented the formation of viable seed.

PHYSIOLOGIC FORMS OF RUST IN THE NURSERY

According to Stakman, Levine, and Bailey (*27*), four distinct physiologic forms of stem rust occur on oats, varying widely in their varietal reaction. Only two of these were found in America. As certain varieties of oats are resistant to one form and susceptible to another, it is imperative in any study of inheritance of rust resistance that the class of reaction to the fungus be known. The results obtained by Stakman and his associates naturally raise the question as to what form was prevalent in the nursery from year to year. Unfortunately, the geographic range of the physiologic forms found in America has not yet been determined. As no effective method, other than the one employed, has been devised to prevent physiologic forms from infecting oat hybrids grown under field conditions, it was necessary to determine which form or forms were present each year. Four methods were used to accomplish this.

In the fall of 1919, a composite sample of *Puccinia graminis avenae* was taken to the greenhouse and maintained in stock culture in the urediniospore stage (*21*). This culture of rust was used to start the initial field infection in the spring of 1920. In the fall a composite sample of rust again was taken from the nursery and used as inoculum on nine pure-line parents. If these parents responded in the same manner to the 1919 and 1920 cultures, the 1919 culture was discarded and the later culture used in the field the next spring. This process was continued throughout the study.

As an additional test for the physiologic response of *Puccinia graminis avenae*, 100 pure-line varieties of oats were grown adjoining the oat-breeding nursery each year, every tenth row being the same pure-line control. These indicated the uniformity of the epidemic.

As already described, the initial infection was started each spring in this nursery with the same culture of *Puccinia graminis avenae* that had been overwintered in the greenhouse. During this five-year period, all varieties showing resistance in 1919 were resistant in the following four years and those susceptible in 1919 were uniformly susceptible thereafter. Moreover, each pure-line parent sown in the breeding nursery showed similar results each year from 1919 to 1923. The results thus far indicate either that only one physiologic form was present in the nursery during the entire time or that these pure lines did not act as differential hosts.

To identify the physiologic form employed in this investigation, the differential hosts of Stakman, Levine, and Bailey (*27*) were exposed to infection. A composite sample of stem rust from the oat-breeding nursery was taken to the greenhouse in July, 1923, and White Tartar, Monarch (C. I. 1760), and the awnless Monarch Selection of Etheridge (C. I. 1879) were inoculated. Monarch Selection of Etheridge (C. I. 1879) was susceptible and White Tartar resistant. According to Stakman and others (*27*), Monarch Selection (C. I. 1879) reacts as a differential host for forms 1 and 2, being resistant to the former and susceptible to the latter. These results suggest that only form 2 (*27*) was present, as Monarch Selection bore only one type of infection, which was normal, with numerous large, coalesced uredinia.[6]

HYBRID VIGOR OF F_1 PLANTS

In crossing certain varieties of oats, remarkable hybrid vigor, expressed as yield and height, was shown by the F_1 plants. (Fig. 2.) Some of these F_1 plants produced more than 2,200 seeds and varied in yield from 10.5 to 36 gm. The parents of these crosses were grown in the same year in field rows adjacent to the F_1 hybrids, but were greatly inferior in yielding capacity. In another cross, Richland (Iowa No. 105), the highest yielding parent, produced only about one-fifth as much as the lowest yielding hybrid of which it and Green Russian were the parents. It should be mentioned that oats make excellent material for genetic studies, as sufficient numbers can be readily obtained in the F_1 generation to make an F_2 inheritance study significant.

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[6] Since the completion of these investigations, it has been shown by Bailey (*2*) that only certain selections of Monarch Selection of Etheridge act as differential hosts for physiologic forms 1 and 2 of *Puccinia graminis avenae*.

The yield in grams and the height in centimeters are the averages of at least 20 parental plants grown under the same cultural conditions as the hybrids. As shown in Table 3, the height in the F_1 did not increase in proportion to the yield, but usually was intermediate between that of the parents. Reciprocal crosses had a response similar to those reported in Table 3.

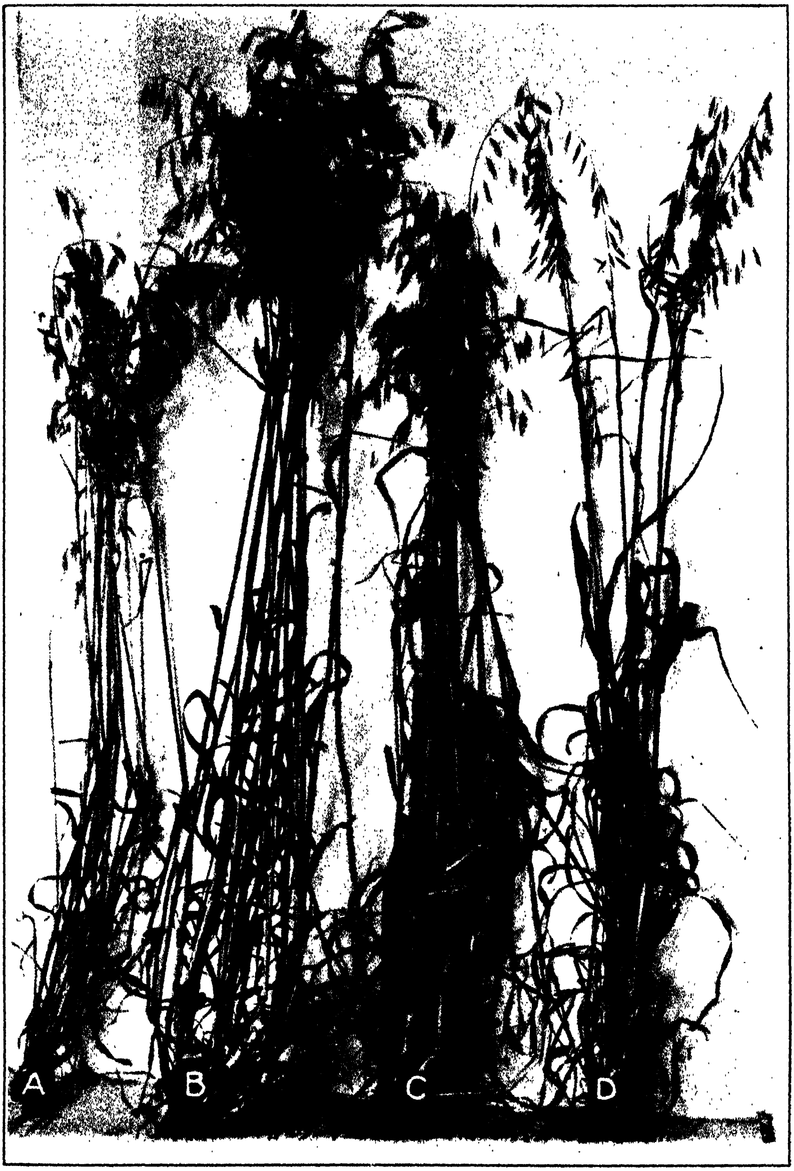

FIG. 2.—Hybrid vigor expressed in height in a White Russian×Burt cross. A, Burt parent; B, F_1 plant; C, F_2 plant; D, White Russian parent

TABLE 3.—*Vigor of parent and F_1 plants of oats in 1919, as expressed by yield and height*

Parents and hybrids	Hybrid No.	Number of plants	Average yield (grams)	Average height (cm.)
White Russian		20	2.7	98
Ruakura		20	1.5	63
White Russian×Ruakura	271	2	22.5	89
Burt		20	2.1	70
White Russian		20	2.7	98
White Russian×Burt	274	4	14.1	90
Richland		20	3.8	79
Green Russian		20	2.6	97
Green Russian×Richland	277	5	21.5	100
Early Ripe		20	1.4	70
Green Russian		20	2.6	97
Green Russian×Early Ripe	280	2	27.5	94
Burt		20	2.1	70
Green Russian		20	2.6	97
Green Russian×Burt	283	3	19.7	91

FACTORIAL EXPLANATION OF RUST RESISTANCE IN OATS

Although rust resistance of cereals has been known to be a heritable character since 1898, when Farrer (*10*) produced resistant wheats by hybridization, the manner of inheritance was not clearly understood until lately. In order to predict the results of a cross between resistant and susceptible oat varieties, the manner of inheritance must be determined. A study of the segregation of the F_2 into resistant or susceptible plants, as verified by the behavior in the F_3, affords the basis for a factorial explanation. An attempt is made to explain the following crosses on a factorial basis.

CROSSES OF RESISTANT AND SUSCEPTIBLE VARIETIES

The first step in the usual method of obtaining material for a factorial explanation of the inheritance of any character involves crossing two individuals differing sharply with respect to this character. After determining the relative reaction of oat varieties to *Puccinia graminis avenae*, crosses were made between resistant and susceptible varieties. Obviously, more can be learned by making the other possible crosses, namely, resistant on resistant and susceptible on susceptible varieties. In this study, hybrids were produced only by crossing resistant with susceptible and resistant with resistant varieties.

RUST REACTION OF THE F_2 PLANTS

If resistance to rust is dominant, all individuals in the F_1 of the cross between resistant and susceptible varieties should be resistant. However, in the present experiments both resistant and susceptible F_1 plants were obtained from crosses involving the susceptible Burt and certain resistant varieties. As it has been shown that all of the 1,115 Burt plants examined were susceptible, this variety must be homozygous for susceptibility to stem rust.

In hybrids obtained from Green Russian × Early Ripe (hybrid No. 280–1 in Table 4), the F_1 was resistant and the F_2 segregated into 254 resistant and 64 susceptible plants. This approximates a 3:1 ratio. In a reciprocal cross (280–2) involving the same parents, the F_2 segregated into 250 resistant to 102 susceptible plants, again approaching a 3:1 ratio. Resistance is dominant and the cross can be explained by the assumption of a single-factor difference. Green

Russian could be symbolized as *SS* (resistant) and Early Ripe as *ss* (susceptible). The F_1, being *Ss*, would be resistant, and the F_2 would segregate in the proportion of 1*SS* : 2*Ss* : 1*ss*, or a 3 : 1 ratio.

TABLE 4.—*Rust reaction of F_1 and F_2 plants from crosses between resistant and susceptible varieties*

Cross	Hybrid No.	F_1		F_2		Ratio		Probable error	$\frac{D}{PE}$
		Resistant (S)	Susceptible (s)	Resistant (S)	Susceptible (s)	Calculated	S:s		
Green Russian×Early Ripe	280-1	S		254	64	238.5:79.5	3:1	±5.21	2.97
Early Ripe×Green Russian	280-2	S		250	102	264:88	3:1	±5.48	2.55
Green Russian×Burt	283-2		s	30	111	26.4:114.6	3:13	±3.13	1.15
Do	283-3	S		179	54	174.8:58.2	3:1	±4.46	.94
White Russian×Burt	274-4		s	23	77	25:75	1:3	±2.92	.68
Do	274-5	S		185	45	174:56	3:1	±4.43	2.48
Do	274-6		s	58	251	57.9:251.1	3:13	±4.63	.02
Do	274-8	S		26	8	25.5:8.5	3:1	±1.70	.29

White Russian × Burt (hybrid No. 274-6, Table 4) was susceptible in the F_1 and in the F_2 segregated into 58 resistant and 251 susceptible plants, thus closely approximating a 3:13 ratio. This cross may be explained on the basis of a two-factor difference, one of which was a resistance inhibitor.

The genotypic composition of the Burt parent in this case can be considered as *ssII*, when *s* = susceptibility and *I* = a resistance inhibitor. The genotype of White Russian, the resistant parent, would be *SSii* where *S* = resistance and *i* = absence of resistance inhibitor. With such an assumption, the F_1 would be *SsIi* or susceptible. The F_2 would segregate in the ratio of 3 resistant to 13 susceptible plants.

The actual results gave a close approximation to the theoretical, *PE* being ± 4.63 and $\frac{D}{PE}$ equaling 0.02.[7] The F_3 results from this cross will be considered in detail in Table 5.

It is apparent from the above hypothesis that three genetically different strains of Burt might be obtained, each of which would breed true for susceptibility and maintain the three genetic compositions, namely, *SSII*, *ssII*, and *ssii*. The fact that these three different genotypes do exist, and breed true for susceptibility in what was thought to be a pure line of Burt, will be shown later. Hybrid No. 274-4, White Russian × Burt (Table 4), was susceptible in the F_1 and segregated into 23 resistant to 77 susceptible plants in the F_2. Fitting this cross to a 1 : 3 ratio resulted in a *PE* of ± 2.92 and $\frac{D}{PE}$ of 0.68. The actual and the theoretical results do not entirely agree, but the deviation probably is not significant. This cross thus can be explained by assuming *SSii* as the genetic composition of White Russian and *SSII* as that of Burt.

In two other crosses of White Russian × Burt (hybrid Nos. 274-5 and 274-8, Table 4), the F_1 plants were resistant. No. 274-5 segregated into 185 resistant and 45 susceptible plants in the F_2. This approximates a 3:1 ratio, the *PE* of which was ± 4.43 and $\frac{D}{PE}$

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[7] *PE* = probable error; $\frac{D}{PE} = \frac{\text{deviation}}{\text{probable error}}$.

equaled 2.48. The F_2 of No. 274–8 segregated into 26 resistant and 8 susceptible plants. Assuming a 3:1 ratio, the *PE* was ± 1.70 and the $\frac{D}{PE}$ 0.29. Both of these could then be satisfactorily explained by assuming *SSii* as the factorial composition of White Russian and *ssii* as that of Burt.

The F_1 derived from a Green Russian × Burt cross was resistant (hybrid No. 283–3, Table 4). The F_2 contained 179 resistant and 54 susceptible plants. Explaining this cross on the basis of a 3 : 1 ratio, the *PE* was ± 4.46 and the $\frac{D}{PE}$ 0.94. Another F_1 plant (hybrid No. 283–2) was susceptible, and the F_2 segregated in the ratio of 30 susceptible to 111 resistant plants. Interpreting this result as representing a 3 :13 ratio, the *PE* was ± 3.13 and the $\frac{D}{PE}$ was 1.15. Green Russian could then be represented as *SSii* and Burt as *ssii*.

It should be pointed out here that Green Russian and White Russian crosses can be explained by assuming the same genetic composition for both of these pure-line parents, while Burt, which breeds true for susceptibility to rust, has at least three different genetic compositions which breed true for susceptibility.

RUST REACTION OF THE F_3 AND F_4 PLANTS

WHITE RUSSIAN×BURT

As shown in Table 4, one cross of White Russian × Burt (hybrid No. 274–6) was susceptible to *Puccinia graminis avenae* in the F_1 generation. The segregation following this cross differed from that of many other crosses made both by Garber (*11*) and by the writer in that the F_2 generation contained 3 resistant to 13 susceptible plants. These F_2 resistant plants showed, on an average, in the F_3 progeny test that one bred true for resistance, while two progenies split in the ratio of 3 resistant to 1 susceptible plant. (Fig. 3.)

Placing these results on a factorial basis, the following factors were assumed: *S* = resistance, *s* = susceptibility, *I* = resistance inhibitor, *i* = absence of inhibitor.

White Russian, then, might be represented as *SSii* and Burt as *ssII*. The following outline would then represent the reaction of the parents and the F_1, F_2, and F_3 generations:

White Russian *SSii* (resistant) × Burt *ssII* (susceptible)

F_1 reaction	F_2 individual plant reaction	F_3 progeny tests
SsIi susceptible.	1 *SSII* susceptible	Homozygous susceptible.
	2 *SSIi* susceptible	1 resistant, 3 susceptible.
	2 *SsII* susceptible	Homozygous susceptible.
	4 *SsIi* susceptible	3 resistant, 13 susceptible.
	1 *SSii* resistant	Homozygous resistant.
	2 *Ssii* resistant	3 resistant, 1 susceptible.
	1 *ssII* susceptible	Homozygous susceptible.
	2 *ssIi* susceptible	Do.
	1 *ssii* susceptible	Do.
	16	

This hypothesis allows a satisfactory explanation of the actual results obtained in this cross of White Russian×Burt (hybrid No. 274–6) as shown in Table 5.

It is well to point out in this connection that the criterion of the whole behavior in this cross is the susceptibility of the F_1 and the two genetically distinct resistant F_2 hybrids, produced in the proportion of one which breeds true for resistance to two which are heterozygous and segregate into three resistant plants to one susceptible plant. A summary of the breeding behavior in the F_3 is presented in Table 5.

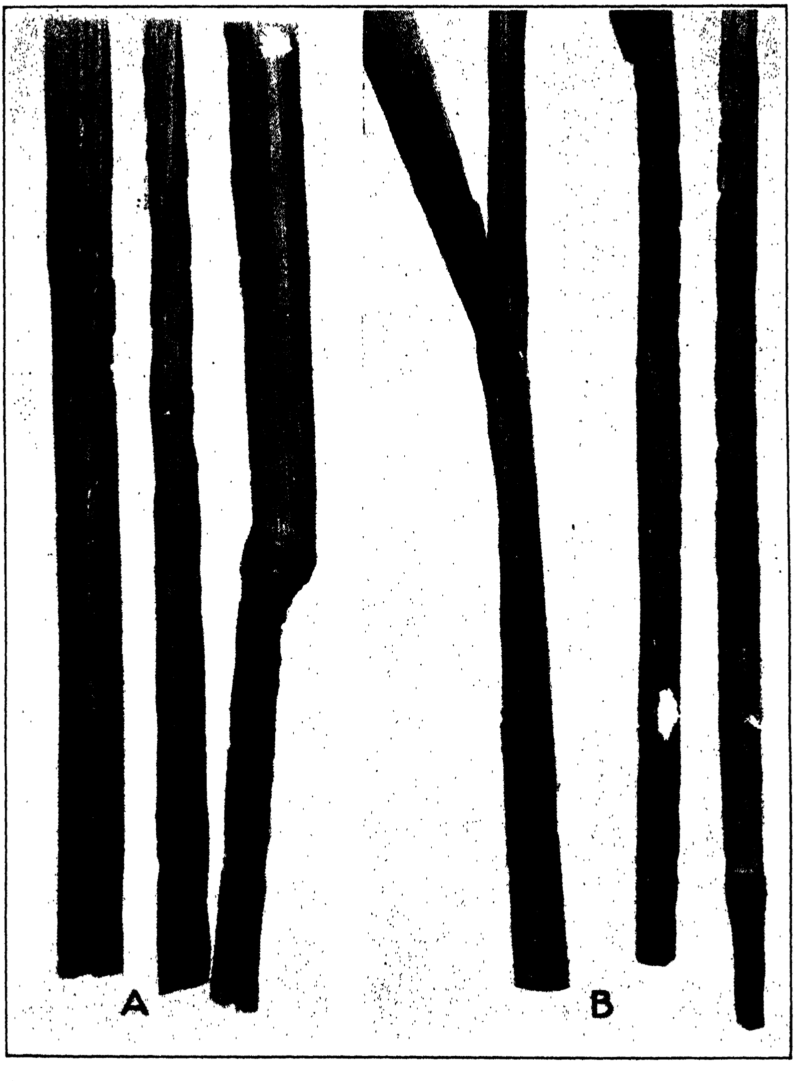

FIG. 3.—Culms from two F_3 plants of a White Russian×Burt cross. These two plants grew side by side in the same nursery row: A, susceptible; B, resistant

TABLE 5.—*Breeding behavior for rust reaction of F_3 families of oats grown from seed of individual F_2 plants of crosses between White Russian and Burt (274-6)*

Breeding behavior in the F_2		Breeding behavior in the F_3									
		Number of homozygous resistant		Number of heterozygous with ratio of—						Number of homozygous susceptible	
				3 : 1			1 : 3 and 3 : 13				
					Plants			Plants			
		Families	Plants	Families	Resistant	Susceptible	Families	Resistant	Susceptible	Families	Plants
58 resistant	Observed	19.0	348	39.0	723	229					
	Calculated	19.3	348	38.7	714	238					
	Deviation	.3		.3	9	9					
251 susceptible	Observed						111.0	748	2,869	140.0	4,755
	Calculated						115.9	753	2,864	135.1	4,755
	Deviation						4.9	5	5	4.9	

The 58 resistant F_2 plants produced 19 F_3 families homozygous for resistance where the expectation was 19.3, and 39 heterozygous where the expectation was 38.7. These latter segregated in the ratio of three resistant to one susceptible plant. The deviation of 0.3 needs no further consideration. The 251 susceptible F_2 plants produced 140 F_3 families homozygous for susceptibility and 111 heterozygous where the expectation was 135.1:115.9. Insufficient plants of the F_3 families were grown to differentiate the 3:13 and 1:3 ratios, respectively.

WHITE TARTAR×NATIONAL

Through the kindness of H. H. Love, of Cornell University, 86 F_2 plants of White Tartar×National (278a) were sent to the writer at Ames, Iowa, in the spring of 1921. Seed from part of these plants was sown that spring and seed from the remaining part in 1923. The National variety is extremely susceptible to stem rust, and White Tartar is resistant. (Fig. 4.) The F_1 and F_2 generations of this cross had been grown at Ithaca, N. Y., where their reactions to stem rust were not recorded. From these 86 F_2 plants, 2,100 F_3 individuals were produced. Of these F_3 families 20 bred true for resistance, 19 bred true for susceptibility, and 47 segregated, producing 854 resistant to 303 susceptible plants. The data for both 1921 and 1923 are summarized in Table 6.

Placing this cross on a factorial basis, White Tartar could be expressed as *SSii* and National as *ssii*. A ratio of 3 resistant to 1 susceptible would be expected in the F_2. In the F_3, 1 F_2 plant should breed true for resistance, 2 should segregate in a ratio of 3 resistant plants to 1 susceptible plant, and 1 should breed true for susceptibility. Explaining this cross on a 3 : 1 ratio, it is found that the resistant F_2 had a PE of ±2.70 and the $\frac{D}{PE}$ was 0.9. The 47 heterozygous progenies splitting into 854 resistant to 303 susceptible plants had a PE of ±9.93 and the $\frac{D}{PE}$ was 1.38.

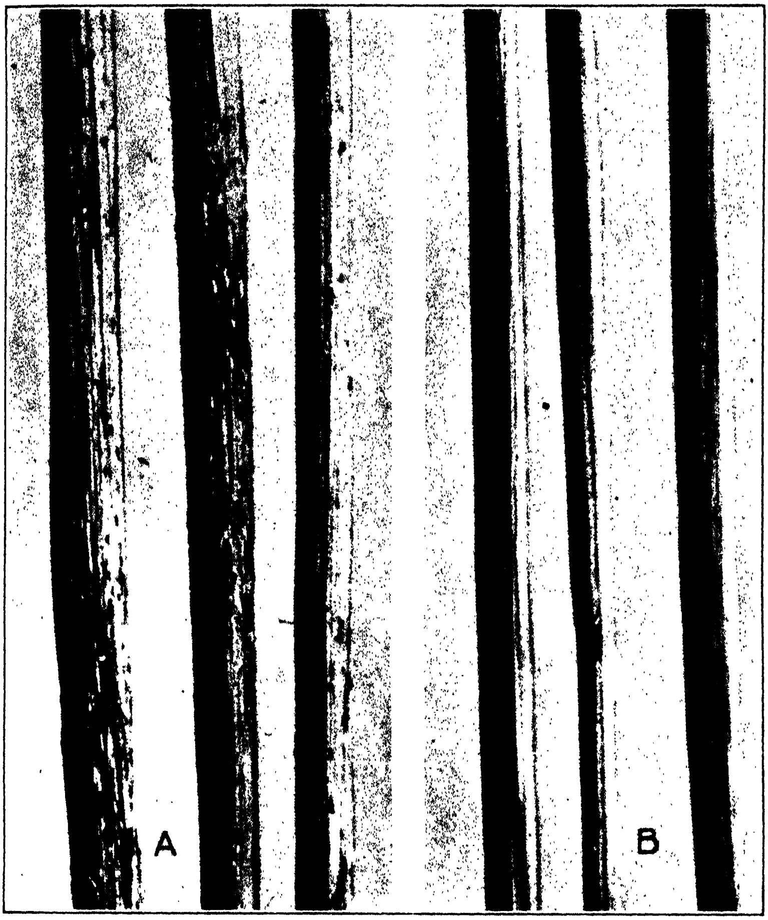

FIG. 4.—Characteristic appearance of susceptible and resistant parents in an oat cross: A, National (susceptible); B, White Tartar (resistant)

TABLE 6.—*Breeding behavior for rust reaction of 86 F_3 families of oats grown from seed of individual F_2 plants of crosses between White Tartar and National (278a)*

Ratio	Breeding behavior in the F_3						
	Number of homozygous resistant		Number of heterozygous			Number of homozygous resistant	
	Families	Plants	Families	Plants		Families	Plants
				Resistant	Susceptible		
Observed	20.0	478	47	854	303	19.0	465
Calculated	21.5	478	43	868	289	21.5	465
Deviation	1.5		4	14	14	2.5	

Assuming a 3:1 ratio, the 86 F_2 plants should have segregated in a ratio of 21.5 homozygous resistant to 43 heterozygous to 21.5 homozygous susceptible families. (Table 6.) The deviation of the calculated from the observed data needs no further comment.

Progenies of 83 plants of the F_3 were studied in the F_4 generation. All plants selected from homozygous resistant and homozygous susceptible F_3 rows bred true for resistance or susceptibility, respectively. Both resistant and susceptible F_3 plants were selected from the heterozygous F_3 rows and their progeny carried through the F_4 generation. These susceptible F_3 plants bred true for susceptibility, whereas two-thirds of the resistant plants segregated in a ratio of three resistant plants to one susceptible, and the other third bred true for resistance.

WHITE TARTAR×LINCOLN

The 175 F_2 plants of the cross White Tartar×Lincoln (253a) were sent to Ames from Ithaca and have the same history as the White Tartar×National. Lincoln is susceptible to stem rust while White Tartar is resistant. (Fig. 5.) From these F_2 individuals 4,405 plants were grown in the F_3. Of the F_3 families, 38, containing 948 plants, were homozygous for susceptibility; 44, containing 1,094 plants, were homozygous for rust resistance; 93, containing 2,363 plants, segregated in a ratio of 1,828 resistant plants to 535 susceptible plants.

Placing these results on a factorial basis, White Tartar could be expressed as *SSii* and Lincoln as *ssii*, where S = resistant and s = susceptible. A ratio of 3 resistant plants to 1 susceptible would be expected in the F_2. Assuming a 3:1 ratio, the deviation between the calculated and observed data is small. (Table 7.)

TABLE 7.—*Breeding behavior for rust reaction of 175 F_3 families of oats grown from seed of individual F_2 plants of crosses between White Tartar and Lincoln (253a)*

Ratio	Breeding behavior in the F_3						
	Number of homozygous resistant		Number of heterozygous			Number of homozygous susceptible	
			Families	Plants			
	Families	Plants		Resistant	Susceptible	Families	Plants
Observed	44.0	1,094	93.0	1,828	535	38.0	948
Calculated	43.7	1,094	86.5	1,773	590	43.7	948
Deviation	.3	----------	6.5	55	55	5.7	----------

In 1922, 101 F_3 plants were studied for their reaction to rust in the F_4 generation. Of these 101 plants, 26, selected from a homozygous resistant F_3 progeny produced 342 F_4 plants, all of which were resistant. Eight susceptible F_3 plants, selected from homozygous susceptible F_3 progenies, bred true for susceptibility in the F_4 generation by producing 107 susceptible plants to none that was resistant.

Theoretically, there should have been three different kinds of plants in the heterozygous F_3, namely, two kinds of resistant and one susceptible. From the segregating F_3 families 43 resistant plants

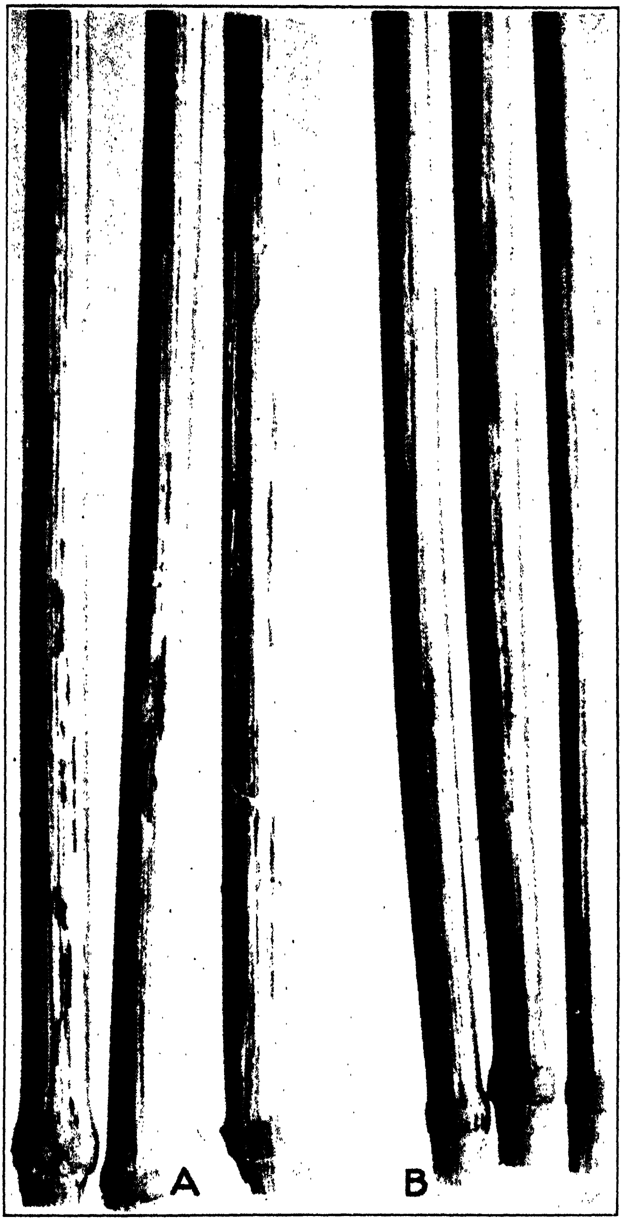

FIG. 5.—Characteristic appearance of susceptible and resistant parents in an oat cross: A, Lincoln (susceptible); B, White Tartar (resistant)

were selected. Of this number, 12 bred true for resistance, producing 177 plants, and 31 segregated in the ratio of 313 resistant to 111 susceptible. The *PE* here, assuming the 3: 1 ratio characteristic of a monohybrid, is ±6.01 and the $\frac{D}{PE}$ is 0.831. Of the susceptible plants selected from the F_3, 24 bred true for susceptibility in the F_4, producing 291 plants. These F_4 families conform to expectation.

CROSSES OF RESISTANT VARIETIES

Rust Reaction of the F_2 and F_3 Plants

In order to study further the inheritance of resistance to stem rust, crosses were made between the two resistant varieties, Green Russian and Richland. As shown earlier (7), Richland possesses inherent resistance to stem rust. Additional crosses were made between White Russian and Ruakura. The latter parent was resistant, but less so than White Russian.

GREEN RUSSIAN×RICHLAND

The F_1 plants of the cross between Green Russian and Richland were more resistant than either parent. In fact, only a few small rust pustules were found. The F_2 plants, as shown in Table 8, segregated with many resistant plants and only a few susceptible ones. Here, again, the resistant plants were more resistant than either parent. The susceptible plants were severely attacked. Hybrid No. 277-4 produced 309 resistant plants to no susceptible ones in 1922, but the remnant seed of this cross produced 186 resistant and 3 susceptible plants in 1923.

Another cross of Green Russian × Richland (hybrid No. 277-3) was studied in the F_3 in 1922 and in 1923. The one susceptible F_2 plant produced 13 susceptible plants in 1922 and 99 susceptible plants in 1923. The 1,000 F_4 plants obtained from the 1922 F_3 susceptible plants all bred true for susceptibility in 1923. In 1922, the progenies of 333 resistant F_2 plants segregated into 244 which bred true for resistance to 89 which segregated. As only 20 seeds from each F_2 plant were sown for the F_3 population, it is probable that 244 resistant plants to 89 susceptible do not express the true ratio.

TABLE 8.—*Reaction to Puccinia graminis avenae of the F_1 and the F_2 plants from crosses between two resistant varieties of oats, Green Russian and Richland*

Hybrid No.	F_1 reaction	Number and reaction of F_2 plants	
		Resistant	Susceptible
277-1	Resistant	322	0
277-2	do	346	0
277-3	do	265	1
277-4 (1922)	do	309	0
277-4 (1923)	do	186	3
277-5	do	256	0

The possibility of a mechanical mixture as an explanation of the behavior of this cross is eliminated by the fact that distinct segregation for height of plant as well as color of the lemma occurred. In addition, the resistant hybrids possessed a more marked resistance than either parent, and the susceptible hybrids were intermediate between the two parents in height of plant.

WHITE RUSSIAN×RUAKURA

Both White Russian and Ruakura were classified as resistant, but the latter consistently showed more stem rust. All of the F_1 plants of the four families carried through the F_2 were resistant, as shown in Table 9. The crosses of these parents responded similarly to those of Green Russian × Richland.

TABLE 9.—*Reaction to Puccinia graminis avenae of the F_1 and F_2 plants from crosses between two resistant varieties of oats, White Russian and Ruakura*

Hybrid No.	F_1 reaction	Number and reaction of F_2 plants	
		Resistant	Susceptible
271-1	Resistant	245	1
271-2	do	100	0
271-3	do	335	5
271-4	do	154	0
Total		834	6

A total of 834 resistant to 6 susceptible plants was produced by all four families. Without doubt the White Russian × Ruakura crosses were hybrids, as evidenced by the typical monohybrid segregation for shape of panicle in the F_2 generation and also by the segregation for height of plant.

No attempt was made to give a factorial explanation of the results obtained by crossing resistant on resistant varieties, as it is probable that insufficient numbers were obtained to express the true ratios in the F_3. It is probable, however, that factors in addition to those considered in this paper are involved in the inheritance of rust resistance in the Green Russian × Richland and the White Russian × Ruakura crosses.

SUMMARY

Out of 770 pollinations, no hybrids were obtained from pollinations made between 11.30 o'clock a. m. and 2.30 o'clock p. m. under field conditions in central Iowa and northern Wisconsin. Pollination at the time of emasculation resulted in some selfed seeds, whereas pollination 24 to 72 hours after emasculation produced true hybrids and a percentage of fertility higher than that resulting after longer periods. Dusting the stigma with pollen produced a percentage of hybrids higher than by inserting the whole anther between the lemma and palea.

Marked hybrid vigor as expressed by yield was obtained in the F_1 generation of oat crosses.

Large uredinia were directly correlated with susceptibility to *Puccinia graminis avenae.*

Resistance to *P. graminis avenae* is dominant and due to a single-factor difference in White Tartar × National and White Tartar × Lincoln crosses.

At least three genetically different strains of Burt were found to breed true for susceptibility. One of these carried a factor which was an inhibitor of resistance. In a Burt × White Russian cross, the F_1 generation was susceptible, the F_2 segregated in the ratio of 3 resistant to 13 susceptible plants. In the F_3, these resistant F_2 plants produced 1 homozygous resistant progeny to 2 progenies which segregated in the proportion of 3 resistant plants to 1 susceptible. In another White Russian × Burt cross the F_1 generation was resistant and the F_2 generation segregated in the ratio of 3 resistant plants to 1 susceptible plant. In still another White Russian × Burt cross, the F_1 generation was susceptible and the F_2 segregated in the proportion of 1 resistant to 3 susceptible plants.

In crosses between resistant varieties such as Green Russian × Richland and White Russian × Ruakura, the F_1 plants were resistant. The F_2 segregated and produced some plants which were more resistant than either parent. It is probable that other factors than the two considered here are involved in the inheritance of resistance to *Puccinia graminis avenae*.

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THE OCCURRENCE OF NOSE SPOTS AND TAIL SPOTS IN GUINEA PIGS[1]

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SCOPE OF PAPER

In the stock of guinea pigs maintained by the Bureau of Animal Industry for experimental purposes, it has often been observed among piebald animals that the nose is one of the portions most likely to be white. Many piebald animals have been recorded, however, which have the nose partly or entirely pigmented. The extent of the pigmented region varies from a small triangular area covering the tip of the nose and extending up to a point between the eyes to an area covering one side of the head and extending over the mid line, or even covering the whole head. It has been observed that this peculiar pattern occurs more frequently in one of the inbred families (family 2) than in the others. In 1918 a selective experiment was started to determine whether the nose-spot pattern could be fixed or increased. Nose-spot animals from family 2 were mated in an experiment called 2N. The present paper deals with the results of this experiment and of the various inbred and crossbred lines in which the nose-spot character occurs.

OCCURRENCE OF NOSE SPOT

Studies were made of the five existing inbred families and of the crossbreeding and control experiments to determine, if possible, the nature and inheritance of the pattern. As is shown in Table 1, the average occurrence of nose-spot animals in four of the inbred families (13, 32, 35, and 39) is 1 per cent, while family 2 shows 9.2 per cent. The two lines of family 2 also differ widely in the percentage of nose-spot animals which they produce; line 2-8-4,[2] which has produced 43.7 per cent of the young born in family 2, produced 14.4 per cent of nose-spot animals, while line 2-9-7, which has produced 36.4 per cent of the young born in this family produced but 3.3 per cent of nose-spot animals. The earlier lines of family 2, producing 18.1 per cent of the animals of this family, produced 9.6 per cent nose spot, practically the same as the average for the whole family. The selection experiment 2N has produced 14.2 per cent of nose-spot animals, which is practically equal to the number produced by the high-producing line of family 2. The crossbreeding experiments, those designated by C-O, CC, BX, etc., show considerable variation in the percentage of nose spot produced, but a study of these variations shows them to be of no statistical significance.

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[2] The symbols 2-8-4 designate the family, generation, and mating to which any animal belongs or from which it has descended. Thus, the above combination means inbred family 2, eighth generation of brother-sister mating, and mating No. 4 of this generation.

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TABLE.—*Total number of animals and number and percentage of nose-spot animals in various lines of guinea pigs*

Stock	Total number of animals	Number of nose-spot animals	Per cent of nose-spot animals
Family 2	4, 148	383	9. 23
Family 13	5, 118	34	[a] . 66
Family 32	3, 695	39	[a] 1. 06
Family 35	3, 806	46	[a] 1. 21
Family 39	1, 978	29	[a] 1. 47
B	5, 697	53	. 93
C-O	1, 304	41	3. 14
C-1	108	7	6. 48
C-2	35	2	5. 71
CC	78	5	6. 41
CY-O	193	5	2. 59
CY-1	501	6	1. 20
CY-2	171	6	3. 51
BX	481	8	1. 66
AC	437	14	3. 20
CA	328	11	3. 35
2G and 2L	38	5	13. 16
Early lines of family 2	752	72	9. 57
Line 2-8-4	1, 812	261	14. 40
Line 2-9-7	1, 510	50	3. 31
2N	648	92	14 20

[a] The average of families 13, 32, 35, and 39 is 1.014.

DIVISION OF FAMILY 2 INTO LINES[3]

In the development of any family from a common ancestor, different lines will gradually be formed due to differences in death rate, rate of reproduction, rate of mating, and other causes. Some lines will become stronger than others and persist, while the weaker ones will become extinct. One line may possess certain hereditary traits differing from another, so that after a few generations it becomes difficult to recognize any relationship between the two lines in question. This has taken place in family 2. A genealogical diagram of this family (fig. 1) shows that throughout its history new branches frequently appeared. Some of these soon became extinct, while others gave rise to numerous other branches, some of which persist to the present day. In this way, two large lines of family 2 have been formed, designated as lines 2–8–4 and 2–9–7. These trace to a common subline, 2–6–8. In the data dealing with the sublines of family 2, 2–9–13 has been included with Group 2–8–4, for the grandparents of 2–9–13 are the parents of 2–8–4. Group 2–9–4 has been included in Group 2–9–7, for both have the same parentage. Male 1 line is slightly different from the other lines of the family in that the foundation sire was mated to two of his daughters. This line was not carried far and only 74 animals were produced.

The early lines of family 2, including all lines up through those starting at the eighth generation except 2–8–4, produced a total of 752 animals, of which 72 had nose spots, giving a percentage of 9.6 and agreeing closely with the family average, 9.2 per cent. At this point a permanent separation occurred. Mating 2–8–4 gave rise to a large group which has produced 1,812 individuals with 261 nose spots among them, or a percentage of 14.4. Likewise, mating 2–9–7 gave rise to another large group producing 1,510 individuals with but 50 nose spots, a percentage of 3.3, which is far below the average for the whole family. If the several small sublines which make up the large groups are examined, considerable variation in the percentage of nose-spot animals produced will be noted and it will be seen that sublines of Group 2–8–4 are consistently much higher than the sublines in Group 2–9–7, with the exception of subline 2–9–4, which produced 19.4 per cent of nose spots. Had this line persisted it might have given rise to a group producing more nose spots even than line 2–8–4. A similar rise in percentage of nose-spot animals produced appears to be taking place in sublines 2–15–6, with 21.7 per cent nose spots, and in 2–18–1, with 26.5 per cent. All lines tracing to mating 2–9–7 have been consistently low producers of nose-spot animals, some rather large sublines not having produced a single nose spot.

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[3] Studies of each of the five inbred families now in existence have been made in detail and it has been found that each family is divided into lines, some quite noticeably different in various respects from others. These differences were especially noticeable in the nose spot and tail spot of family 2, otocephaly in family 13, and polydactyly in family 35.

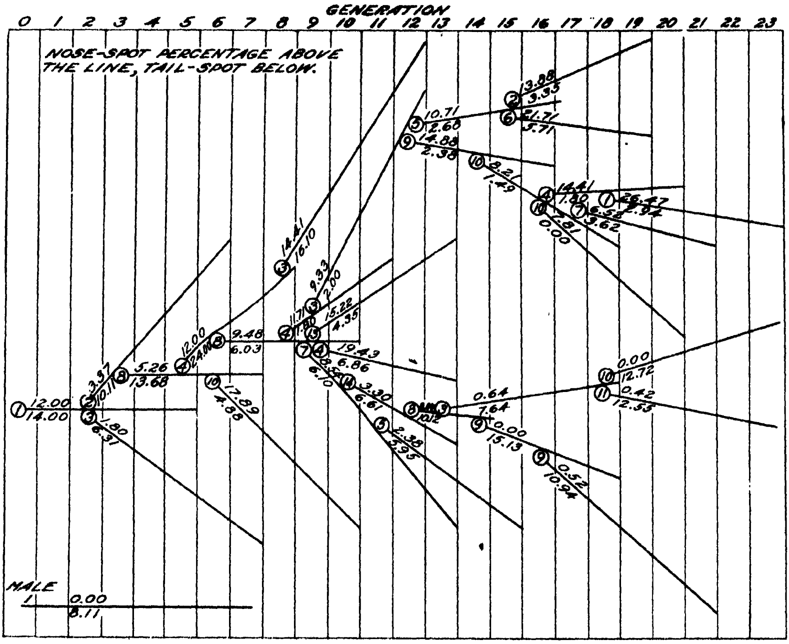

FIG. 1.—Lines of family 2 and percentage of nose-spot and tail-spot animals in each

It is interesting to note that there is also a difference in these two large groups of family 2 in respects other than nose spot. In detailed studies of the inbred families of guinea pigs a difference in the amount of pigment in the coat of some lines was found. Group 2–8–4 showed a low percentage of white—55.4 per cent for males and 68.9 per cent for females—while 2–9–7 showed 82.7 per cent for males and 87.7 per cent for females. This difference can not be accounted for by the presence of the nose spot. In arranging the normal and nose-spot animals in the two lines of family 2 and experiment 2N according to the distribution of white, one sees that there is very little difference between the nose-spot animals and those without the nose spot within the same line, the difference being for the 2–8–4 line about 2.5 per cent, for the 2–9–7 line and for 2N about 5 per cent. Males show a slightly larger difference than females. When all groups are considered together, the males show a difference of about 4.5 per cent white between animals with nose spot and those without it; and females a difference of about 2.8 per cent. These differences and the range of white are shown graphically in Figure 2.

These differences can be accounted for by the nose spot, as will be seen in an explanation of the grading system used. At birth, a rubber-stamp outline of the dorsal and ventral surfaces of the guinea pig is made and filled in by vertical or horizontal lines to represent the extent of differently colored areas, black being shown by vertical lines, and red by horizontal lines. A transparent pattern of exactly the same outline is divided into 10 areas of equal size for the dorsal pattern and 8 for the ventral; the feet and legs are counted as 2 divisions, thus making the whole surface of the animal divided into 20 areas, each representing 5 per cent of the area of the animal. The percentage of color of each animal is determined by laying the transparent divided pattern over the diagram of the animal in question, and recording the number of parts of color on the dorsal and ventral

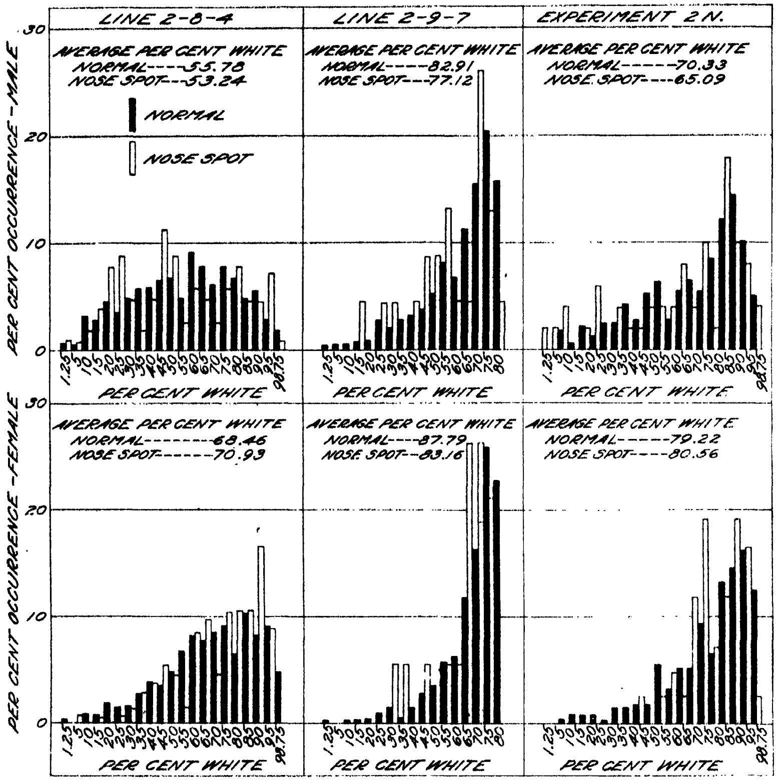

FIG. 2.—Percentage of white and frequency of occurrence of normal and nose-spot animals in family 2 and experiment 2N

surfaces separately. These parts can readily be converted into percentages by multiplying by five. In general, the pigmented area on the nose occupies one of these divisions, equaling about 5 per cent of color; thus a nose-spotted animal would show approximately 5 per cent less of white than an animal with an unpigmented nose.

The size of the litter is slightly smaller in line 2–9–7 (2.39 ± 0.026) than in 2–8–4 (2.41 ± 0.023), the difference being 0.02 ± 0.035. Along with differences in litter size there is also a difference in weight at birth and at weaning, these being for the 2–8–4 line, 72.283 ± 0.277 and 179.066 ± 0.810 gm., respectively, and for the 2–9–7 line 74.732 ± 0.317 and 180.441 ± 0.946 gm., respectively. The differences between the two lines are slight, 2.449 ± 0.421 gms. for birth-weight difference and 1.375 ± 0.246 gm. for weaning weight. By using a correction factor to get the weights all on the basis of an average size of litter, the average litter size being regarded as 2.5, the birth weight for the two lines under consideration becomes 71.56 gm. for the 2–8–4 and 73.76 gm. for the 2–9–7 line. Weaning weights are 177.63 gm. and 178.64 gm., respectively. The difference still persists and is but slightly reduced by the correction factor.

These several differences between two large groups of family 2 indicate some distinct differences in their genetic constitution which are transmitted from generation to generation, and in the case of nose spotting seems to give rise to higher-producing lines at certain points in the history of the family. This condition agrees with the results found in family 13 as regards the production of otocephaly (*7*).[4] Throughout the history of this family a deformity known as otocephaly has appeared. One and fifty-four hundredths per cent of the animals born were of the otocephalic type. At various points in the history of the family a rise in the percentage of otocephaly occurred, and finally, in the thirteenth generation, two distinct lines separated, one producing 2 per cent of otocephali, the other 8.9 per cent. Even in this high-producing line certain sublines produced as high as 21.5 per cent of otocephalic young, suggesting the possibility of still higher producing lines in future generations.

RESULTS OF CROSSING

The first cross between the various inbred families has been designated as C–O. Only crosses in which the present five families are involved have been included in the data of this paper. C–1 is composed of brother-sister matings from C–O, and C–2 of brother-sister matings from C–1, so that both involve only two inbred families. CC is a cross between two C–O matings, and therefore usually involves four inbred families. BX is a back cross, a C–O mated back to one of the parental inbred lines. AC and CA involve three inbred families, the C parent being from a C–O mating and the A parent being an animal from one of the inbred families. The CY experiment differs from the C experiment in that only family 2 and a certain line of family 13 are involved. 2G and 2L are selection experiments from family 2, but only a few animals are involved in these experiments. (See Table 1.)

Some of the different experiments are comparable, then, and Table 2 groups them according to their similarity, and whether the family 2 parent came from the high or low nose-spot-producing line. Since family 13 produced the fewest nose spots, crosses between this family and family 2 and the F_2 and F_3 young of these crosses have been grouped separately, for the purpose of studying segregation. While the data presented show that the high line of family 2 does produce more nose spot in crosses with family 13 than the low line, the differences are not statistically significant. This difference is in the reverse direction in crosses with the other inbred families. Comparisons between succeeding generations of the cross between families 2 and 13 show in general a rise of the percentage of nose spot in the F_2 and F_3 generations, but here again the differences are statistically not significant.

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[4] Reference is made by number (italic) to "Literature cited," p. 41

A tabulation of the various crosses with respect to the percentage of family 2 ancestry shows also very little difference in the percentage of nose spot produced. Animals which have one-quarter, one-half, or three-quarters of the blood of family 2 show very little difference in the percentage of nose spot produced.

TABLE 2.—*Number and percentage of nose-spot young in crosses of family 2 with other inbred families*

Family 2 crossed with—	Experiment	High nose-spot line of family 2: Total number of young	High nose-spot line of family 2: Number of nose-spot young	High nose-spot line of family 2: Per cent of nose-spot young	Low nose-spot line of family 2: Total number of young	Low nose-spot line of family 2: Number of nose-spot young	Low nose-spot line of family 2: Per cent of nose-spot young	Difference
Family 13	C-0	134	5	3.73	235	2	0.85	
	CY-0	120	3	2.50	73	2	2.74	
Total F_1		254	8	3.15± .739	308	4	1.30± .435	1.85±0.858
Family 13	C-1	8	0	0	37	4	10.81	
	CY-1	263	6	2.28	238	0	0	
Total F_2		271	6	2.21± .602	275	4	1.45± .486	.76± .774
Family 13	C-2				14	1	7.14	
	CY-2	104	5	4.81	67	1	1.49	
Total F_3		104	5	4.81±1.415	81	2	2.47±1.163	2.34±1.832
2×13 difference:								
Between F_1 and F_2				.94± .954			.15± .652	
Between F_1 and F_3				1.66±1.597			1.17±1.242	
Between F_2 and F_3				2.60±1.538			1.02±1.261	
Family 32	C-0	128	3	2.34	189	4	2.11	
Family 35	C-0	166	5	3.01	104	7	6.73	
Family 39	C-0	75	3	4.00	105	6	5.71	
Total of above, 3 crosses.		369	11	2.98± .597	398	17	4.27± .684	1.29± .908

PERCENTAGE OF NOSE-SPOT YOUNG CLASSIFIED BY FAMILY 2 ANCESTRY

Ancestry	Total number of young	Number of nose-spot young	Per cent of nose-spot young
One-fourth blood, family 2	1,286	35	2.722±0.306
One-half blood, family 2	2,312	67	2.898± .235
Three-fourths blood, family 2	38	3	7.895±2.950
Difference:			
Between one-fourth and one-half bloods, family 2			.176± .386
Between one-fourth and three-fourths bloods, family 2			5.173±2.966
Between one-half and three-fourths bloods, family 2			4.997±2.960

RESULT OF SELECTION FOR NOSE SPOT

In 1918 a selection experiment called 2N was started for further study of the nose-spot character. Animals from family 2 possessing a nose-spot were mated among themselves regardless of their relationships. The animals used were not separated as to generations, but any showing nose spot were mated. This experiment is still in progress. Through December, 1926, there had been 648 animals produced with 92 nose-spots, a percentage of 14.2 which is practically equal to the percentage in the nose-spot-producing lines of family 2. There is much similarity between 2N and family 2 in weight of individuals, size of litter, mortality, and amount of white. The percentage of males is somewhat lower in 2N than in family 2, being 46.42 and 50.37 per cent, respectively. Differences in these experiments are given in Table 3.

TABLE 3.—*Difference in various characters between the lines of family 2 and experiment 2N, and between nose-spot animals and litter mates without nose spot*

Line or experiment	Birth weight	Size of litter	Per cent born alive	Per cent raised of those born alive	Per cent of all young raised to weaning	Per cent white		Sex ratio (per cent males)
						Male	Female	
	Grams	*Number*						
2-8-4	72.283	2.41	80.545	83.399	67.174	55.37	68.89	51.259
2-9-7	74.732	2.39	83.279	83.918	69.886	82.72	87.66	52.306
Total family 2	74.565	2.39	81.768	84.257	68.895			50.374
Nose-spot animals from family [illegible]	66.700	2.72	86.680	83.130	72.060	56.89	72.44	51.060
Litter mates not showing nose spots	66.050	2.68				60.54	75.55	
Total 2N	70.380	2.49	82.250	84.240	69.290	69.42	78.66	46.420
Nose-spot animals from experiment 2N	69.390	2.80	80.430	87.840	70.650	65.08	80.56	54.350
Litter mates not showing nose spots	68.280	2.81				71.75	82.85	

It is of interest to study 2N according to the ancestry of the parents as regards nose spot. This study can be made in two ways. One is by classifying the young as to whether one, or both, or neither parent was from the high-producing nose-spot line of family 2. By this method of classification it was found that when both parents were from the low-producing nose-spot line 10.9 per cent of the young had a colored nose; when one parent was from the high-producing line and the other from the low, 12.2 per cent had a nose spot, and when both parents were from the high-producing line 14.9 per cent had nose spot. (Table 4.) The numbers entering into these percentages are small, the remainder of the animals of this experiment having been produced by parents of mixed ancestry from those original matings directly from family 2. Although there is a gradual rise in the percentage of nose-spot animals produced, the differences between the groups are not significant.

The second way in which 2N may be studied is by classifying the young as to the number of nose-spot ancestors they had for three generations back, i. e., to the great-grandparents. It is seen that in cases where neither parent nor any of the grandparents or great-grandparents had nose spot, a smaller percentage of the young had nose spot; the average being slightly over 9 per cent when parents or grandparents are considered, and nearly 7 per cent in the case of great-grandparents. When one or more of the parents, grandparents, or great-grandparents had a nose spot, a larger percentage of the young also had this character, but this increase is not consistent with the increase of nose-spot ancestors.

TABLE 4.—*Amount of nose spot in 2N classified by ancestral family 2 lines*

Ancestry	Total number of young	Number of nose-spot young	Per cent of nose spot young
Both parents from low nose-spot line of family 2	46	5	10.87±3.094
1 parent from high nose-spot line of family 2	82	10	12.20±2.4378
Both parents from high nose-spot line of family 2	67	10	14.92±2.9359
Difference:			
Between Groups 1 and 2			1.33±3.9401
Between Groups 1 and 3			4.05±4.2662
Between Groups 2 and 3			2.72±3.8160

AMOUNT OF NOSE SPOT IN 2N CLASSIFIED BY NUMBER OF NOSE-SPOT ANCESTORS

Relationship	Number of nose-spot ancestors								
	0	1	2	3	4	5	6	7	8
Parents:									
Total number of young	32	59	557						
Number of nose-spot young	3	12	77						
Per cent of nose-spot young	9.38	20.31	13.82						
Grandparents:									
Total number of young	53	87	105	114	289				
Number of nose-spot young	5	12	12	21	42				
Per cent of nose-spot young	9.43	13.79	11.43	18.42	14.53				
Great-grandparents:									
Total number of young	29	21	111	111	63	22	132	6	153
Number of nose-spot young	2	2	16	14	12	3	21	1	21
Per cent of nose-spot young	6.90	9.52	14.41	12.61	19.05	13.64	15.91	16.67	13.72

In family 2 and in experiment 2N a selective process has been going on, in the case of family 2 unconsciously, in 2N consciously. As family 2 was subdividing into lines, gradually the frequency with which nose-spot animals were produced increased in certain matings and in the lines arising from these matings. Thus, there gradually arose the one line producing over 14 per cent of nose-spot animals and the other line producing only a little more than 3 per cent. Since family 2 has been maintained wholly by brother-sister matings, all matings have been made at random so far as the nose-spot character is concerned. In this case the similarity between the mated animals has been primarily genetic and only incidentally somatic. In 2N the matings have been made on somatic resemblance incidentally genetically similar because of the several generations of close inbreeding behind them. This accounts for the great similarity in family 2 and 2N in respect to weight, mortality, and amount of white.

In discussing the effects of selection, Wright (*3*) says that random mating may be followed in a selected line without loss of progress toward homozygosis. Selection of heterozygotes leads to no fixation of characters. After an indefinite number of generations of selection a return to random breeding brings the population again to an unselected condition. Almost the full effect of selection is reached in the first generation if the average of the parents is of the desired type, and further selection merely reduces the variability. The rate at which characters can be fixed is much increased by combining inbreeding and assorted mating based on somatic resemblance. These statements seem to explain why 2N does not show a higher percentage of nose spot than the nose-spot lines of family 2. In reference to genetic similarity, family 2 has been selected, but in relation to nose spot the matings were made at random. The whole family 2 population, therefore, represented an unselected condition, but one line of this family, through unconscious selection, was already of a certain desired type. Then, when further selection was made from this type for experiment 2N, no increase in the nose-spot character resulted. The rapidity with which 2N showed results comparable with the 2-8-4 line is due to the combined inbreeding and selection.

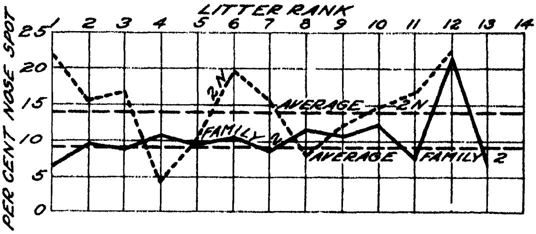

FIG. 3.—Percentage of nose spot by litter rank

RELATION OF NOSE SPOT TO OTHER CHARACTERS

LITTER RANK AND SEASON OF BIRTH

In other studies (*4*, *5*, *6*) of the guinea-pig experiments, certain relationships between different characters were found. A study of some of these characters has been made to determine whether there is any relationship between them and nose spotting. The first character studied was litter rank. Tabulations were made to determine the percentage of nose-spot guinea pigs produced in succeeding litters. A slight tendency for the percentage to rise in later litters of family 2 was noted. The percentage in 2N, however, fluctuated considerably, due to small numbers, but in general it followed close to the average line. This relationship is shown in Figure 3. The percentage of nose-spot young born in any month shows a close agreement with the average. Again, 2N shows much more variation than family 2. (Fig. 4.)

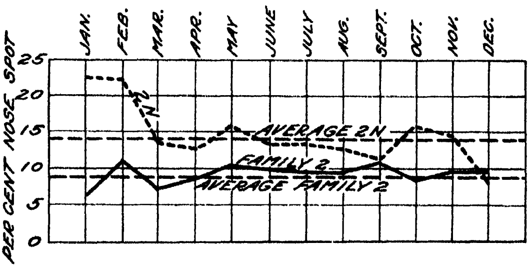

FIG. 4.—Month of birth of nose-spot guinea pigs

SIZE OF LITTER

In determining the size of litters in which nose-spot guinea pigs were born, the individual, rather than the litter, was taken as the unit, the litters being weighted, therefore, by the number of individuals. This gives a more satisfactory comparison where individuals of a litter are concerned than the litter as a whole. The averages thus obtained are somewhat higher than averages derived from using the litter as the unit.

In family 2 three divisions were made in comparing the size of litters in which nose-spot animals were born with the size of the litters in which their normal brothers and sisters were born. The first division included the early lines of the family before the separation into two distinct lines took place. Nose-spot animals were born in litters averaging 2.72 in size (Table 5), and their normal brothers and sisters in litters of 2.68. Line 2–8–4 nose-spot animals were produced in litters averaging 2.75, while their normal brothers and sisters were born in litters of 2.70. Line 2–9–7 nose-spot animals were produced in litters of 2.54 average size while their normal brothers and sisters were born in litters of 2.60. Family 2 as a whole produced nose-spots in litters of 2.72 average size and their normal brothers and sisters were born in litters of 2.68, a difference of 0.04 ± 0.034. In 2N, nose-spot animals were born in litters of 2.80 average size and their normal brothers and sisters in litters of 2.81. The differences and the probable errors show that nose-spot animals are not born in appreciably larger or smaller litters than their normal brothers and sisters.

TABLE 5.—*Litter and size of nose-spot guinea pigs and of their normal brothers and sisters*

Lines	Size of litter										Total	
	1		2		3		4		5			
	Nose-spot animals	Normal animals	Nose-spot animals	Normal animals	Nose-spot animals	Normal animals	Nose-spot animals	Normal animals	Nose-spot animals	Normal animals	Nose-spot animals	Normal animals
Early lines, family 2	7	32	26	126	25	122	8	24	6	29	72	333
2-8-4	18	91	79	485	118	545	42	226	4	16	261	1,363
2-9-7	5	32	22	126	14	121	9	47	0	5	50	331
2N	5	27	28	180	41	232	16	104	2	13	92	556

Lines	Average size of litter		Difference
	Nose-spot animals	Normal animals	
Early lines, family 2	2. 72± 0. 084	2. 68± 0. 038	0. 046± 0. 092
2-8-4	2. 75± .036	2. 70± .016	.051± .039
2-9-7	2. 54± .086	2. 60± .033	.058± .092
2N	2. 80± .061	2. 81± .025	.009± .066

Since the study of nose spot has involved animals almost wholly from family 2, it will be expected at once that there will be a close similarity to family 2 in weight. Since in other studies of weight in guinea pigs (*4*, *5*), there has been found a high correlation between birth weight and weaning weight, only birth weight has been considered in this study. The average birth weight of all young in family 2 was 74.6 gm. The average birth weight of all nose-spot young in family 2 for all sizes of litters was 70.9 gm.; in 2N it was 69.4 gm. In comparing the weight of the nose-spot animals with the weight of their litter mates without nose spot a close agreement is found. In family 2 the weight of nose-spot animals which had litter mates was 66.7 gm., while the weight of their normal litter mates was 66 gm. In 2N, nose-spot animals weighed 69.4 gm. and their normal litter mates weighed 68.3 gm. The differences are small and not significant.

MORTALITY

The mortality of the guinea pigs used in the investigations of the Bureau of Animal Industry has been measured by three percentages: The percentage of young born alive, the percentage raised of those born alive, and the percentage of all young raised to weaning. These percentages for the two large lines of family 2 and 2N are shown in Table 3. It will be seen that there is a very close agreement in these percentages, indicating that there is little if any relation between nose spot and mortality.

SEX

Sex ratio in these experiments has been expressed as percentage of males. The percentage of males in family 2 as a whole is 50.4 ± 0.524. The percentage of males among the nose-spot young is 51.1 ± 1.734. Experiment 2N as a whole produced 46.4 ± 1.341 per cent males, while among the nose-spot young the percentage of males was 54.4 ± 3.503. These apparently wide departures in 2N are probably due to small numbers as compared with family 2. The difference is 7.93 ± 3.75, which can not be considered statistically significant.

COLOR OF NOSE SPOT AND COAT-COLOR RELATIONS

Family 2 is a tricolor—black, red, and white. In classifying the nose-spot individuals as to color it was found that there was a large excess of animals having red spots. In family 2, out of 383 animals which showed this pattern there were 111 with black nose and 272 with red nose, or 28.98 and 71.02 per cent, respectively. Experiment 2N produced 92 nose spots, 23 black, and 69 red in percentages of 25 and 75, respectively.

To determine whether this relation holds also for other parts of the body a tabulation was made of the color of the right shoulder and left hip. An equal area was observed on each animal by cutting out squares of equal area over shoulder and hip on a transparent pattern which was laid over the original color diagram of the animal. The color that filled the largest portion of this cut-out area was recorded as the color for that particular spot. There were 3,082 animals observed, of which 1,055 had either a black or red right shoulder. In the same group 1,027 had a colored hip.

A further study was made by tabulating the amount of black, red, and white in the two lines of family 2 and comparing the ratio of black and red on the whole coat to the same ratio for a certain area of the coat. The ratio of black to red in the two large lines of family 2 was almost equal, although there is a large difference with respect to the amount of white, as has been mentioned previously. The ratio of black to red is slightly larger with males than with females. The tabulation of shoulder and hip spots was not segregated as to sex, but the ratio of the sexes combined is not greatly different from the ratio for the whole coat. Shoulder spots and hip spots show a greater tendency to red. This tendency to red is still greater in the nose spots, where the ratio for the sexes combined for family 2 is 1: 2.5 and for 2N is 1 : 3. These color relations are shown in Table 6.

TABLE 6.—*Color relations of guinea pigs in lines of family 2 and in experiment 2N*

PER CENT OF COLOR ON WHOLE COAT OF ANIMALS

Lines	Black		Red		White		Ratio black to red	
	Males	Females	Males	Females	Males	Females	Males	Females
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2-8-4	18.364	13.630	27.051	18.766	55.457	68.756	1:1.47	1:1.38
2-9-7	7.722	6.140	11.308	8.246	82.715	87.590	1:1.46	1:1.34

PER CENT OF ANIMALS WITH DIFFERENT COLORED SPOTS, FAMILY 2 AND EXPERIMENT 2N

Part of body	Black	Red	White	Ratio black to red
	Per cent	*Per cent*	*Per cent*	*Per cent*
Right shoulder	13.89	20.34	65.77	1:1.46
Left hip	12.69	20.64	66.68	1:1.63
Nose, family 2	2.68	6.56	90.77	1:2.45
Nose, 2N	3.55	10.65	85.80	1:3.00
Tail, family 2	2.94	3.98	93.08	1:1.35

TYPES OF MATINGS

The matings that have produced nose-spot animals in family 2 and in experiment 2N have been classified as to parentage of the animals into groups according to the color of the nose spot, or whether there was no spot at all. Table 7 shows the percentage of animals of each class born in the different types of matings.

The absence of definite hereditary results may be explained when the genetic factors which determine the color pattern of family 2 are considered. The pattern concerned is known as the tricolor pattern, i. e., it consists of black, red, and white. Two genetic factors are involved, e^p, the factor for partial extension, which determines the presence of red spots on an otherwise solid black coat, or complete extension, E, and s the piebald factor which determines white spots on an otherwise solid colored coat, S. These factors are homozygous ($e^p\ e^p\ ss$) in the inbred stock. They do not, however, determine the amount of color or its location, for the amount of color varies greatly in the two lines of family 2, as has been pointed out before, and even in individuals of the same litter there is great variation. The correlation of amount of white on litter mates of this family is about 0.16 ± 0.026, being about equal in both lines, whether the correlation is between brothers, sisters, or between brothers and sisters.

TABLE 7.—*Types of matings and percentage of young produced, family 2 and experiment 2N*

Type of mating	Number of matings	Family 2					
		Black nose spot		Red nose spot		No nose spot	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Black with black	1	1	4.35	0	0	22	95.65
Black with red	3	4	10.25	4	10.25	31	79.49
Black with normal	11	4	1.91	30	14.35	175	83.73
Red with red	2	2	7.69	3	11.55	21	80.77
Red with normal	38	22	4.71	55	11.78	390	83.51
Normal with normal	107	78	4.46	180	10.29	1,491	85.25
Both parents nose spot	6	7	7.95	7	7.95	74	84.09
One parent nose spot	49	26	3.85	85	12.57	565	83.58
All classes	162	111	4.41	272	10.82	2,130	84.75

Type of mating	Number of matings	Experiment 2N					
		Black nose spot		Red nose spot		No nose spot	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Black with black	3	0	0	4	7.55	49	92.45
Black with red	21	10	3.66	28	10.26	235	86.08
Black with normal	1	2	8.33	4	16.67	18	75.00
Red with red	20	10	4.33	25	10.32	196	84.85
Red with normal	3	1	2.86	5	14.28	29	82.86
Normal with normal	2	0	0	3	9.38	29	90.62
Both parents nose spot	44	20	3.59	57	10.23	480	86.18
One parent nose spot	4	3	5.08	9	15.25	47	79.66
All classes	50	23	3.55	69	10.65	556	85.80

WORK OF OTHER INVESTIGATORS ON NOSE SPOT

MacCurdy and Castle (*2*) in their selection for nose spot obtained somewhat different results from those of the present writer. They increased appreciably the occurrence of nose spot in their stock. From a nose spot male mated with different kinds of females, they obtained 50 per cent of the young with nose spots. In matings of the descendants of this male with nose-spot females, 39.8 per cent of the young had nose spots. In matings in which none of the parents bore a nose spot, 16.3 per cent of the young had nose spots. These experiments showed an increase in the amount of nose-spot young with an increase in the amount of nose-spot ancestry, and strongly suggested the possibility of a still further increase by continued selection. MacCurdy and Castle had not obtained such an increase, however, at the time of the publication of their results. Their results differed further from those obtained in the Bureau of Animal Industry experiment in the amount of white on the animal. In every case the young with nose spots had fewer other spots on the body than the young without nose spots. While the data of MacCurdy and Castle do not take into account the size of the spots, and thus can not be expressed in percentage of white or colored area, the fact that there is a larger average of other colored areas in the case of the animals without nose spot suggests that these animals also have less white than the nose-spot individuals. This is directly opposite to the result found in the Bureau of Animal Industry experiment.

OCCURRENCE OF TAIL SPOT

Another interesting pattern that has been studied along with nose spot is tail spot. This pattern consists of a small patch of color covering the tail area. Animals which have shown white on one side of the mid-dorsal line and color on the other, or which have color extending across the whole rump on both sides have not been included as tail-spot animals. The five inbred families and the animals in experiment B have been included in this study. The percentage occurrence of tail spot is given in Table 8.

TABLE 8.—*Tail spotting in families of guinea pigs*

Stock	Total number of animals	Number of tail-spot animals	Per cent of tail-spot animals
Family 2 (early lines)	752	80	10. 64
2–8–4 line	1, 812	51	2. 81
2–9–7 line	1, 510	150	9. 93
Total, family 2	4, 148	287	6. 92
Total, family 13	5, 118	60	[a] 1. 17
Total, family 32	3, 695	16	[a] .43
Total, family 35	3, 806	69	[a] 1. 81
Total, family 39	1, 978	93	[a] 4. 70
Experiment B	5, 697	229	4. 02

[a] The average for families 13, 32, 35, and 39 is 1.63 per cent.

A condition very similar to that found in the nose-spot study is here shown. Different lines of family 2 show a difference in the percentage of tail spots produced, and the family as a whole produces more than any other of the inbred families, or than occur in the control experiment B. The average for the four inbred families, besides family 2, is 1.63 per cent.

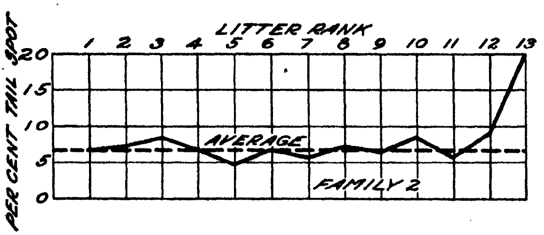

FIG. 5.—Percentage of tail spot by litter rank

In the study of family 2 by lines it has been found that there is a similarity to the nose-spot condition. The early lines produced 10.64 per cent of tail spot animals, or about the average of line 2–9–7. At the point where the two large branches emerge a difference in the amount of tail spotting appears, but the difference is in an opposite direction to that of the nose spotting. Line 2–8–4, which produces the most nose spot produces the fewest tail spots, only 2.8 per cent, and the low nose-spot-producing line, 2–9–7, produces the most tail spots, 9.9 per cent. This rather peculiar result makes it appear as though an animal does not have color on both extremities at once although several animals have been observed that had both nose spot and tail spot. At 2–12–8 a rise in percentage of tail spot is seen, persisting in the other lines that have descended from this mating. This is comparable with the rise in percentage of nose spot animals in the 2–15–6 and 2–18–1 sublines. This 2–12–8 line with the line descending from it produces a percentage of 11.45 tail-spot individuals, while the line 2–9–7 up to the beginning of 2–12–8 produced but 6.49 per cent, showing an increase of nearly 5 per cent. (Fig. 1.)

In studying the relations between tail spot and other characters it has not been considered necessary to go into detail as much as was done in the case of nose spot, for the relations appear practically the same. (Figs. 5 and 6.) Curves showing the percentage of young with nose spot born in a certain month agree very closely with the total percentage of tail-spot young born. Likewise, curves showing the percentage of tail-spot young born in a certain litter compare closely with the total percentage of tail-spot young born. Weights have not been considered, since in the discussion of nose spot it was shown that very little difference existed between the two large lines of family 2. It seems reasonable to expect the same with the tail-spot condition. Likewise, mortality has not been considered.

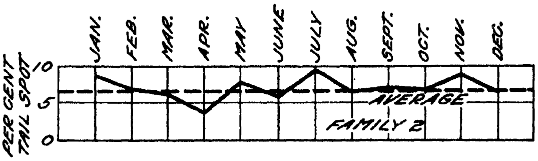

FIG. 6. – Month of birth of tail-spot guinea pigs

The relation between sex of the animal and color of the spot seems rather important to consider.

TABLE 9.—*Number and percentage of male and female guinea pigs having black or agouti and red, yellow, or cream tail spots*

Stock	Guinea pigs having black or agouti tail spots				Guinea pigs having red, yellow, or cream tail spots				Total number of guinea pigs
	Male		Female		Male		Female		
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	
Family 2	59	20.56	63	21.95	80	27.87	85	29.62	287
Family 13	13	21.67	24	40.00	12	20.00	11	18.33	[a] 60
Family 32	4	25.00	7	43.75	1	6.25	4	25.00	16
Family 35	15	21.74	30	43.48	6	8.70	18	26.09	69
Family 39	17	18.28	55	59.14	11	11.83	10	10.75	[a] 93
B	51	22.27	87	37.99	44	19.21	47	20.52	229
Total	159	21.09	266	35.28	154	20.42	175	23.21	754
	Male and female				Male and female				
	Number		*Per cent*		*Number*		*Per cent*		
Total, by color	425		56.37		329		43.63		
	Male (all colors)				Female (all colors)				
	Number		*Per cent*		*Number*		*Per cent*		
Total, by sex	313		41.51		441		58.49		

[a] In family 13 there were 9 males and 9 females which had white tail spots surrounded by a colored area, and in family 39 there were 17 males and 10 females similarly colored. These have not been included in the tabulation.

In family 2 an excess of red tail spots (57.49 per cent red to 42.51 per cent black) was found. This does not approach, however, the excess of reds that was shown in nose spot, the ratio here being 1 : 1.35 for both sexes combined. In the case of sex, there is a considerable excess of females with tail spots (58.49 per cent females to 41.51 per cent males), whereas with nose spotting there was nearly equality between the sexes, 48.30 per cent females to 51.70 per cent males in family 2 and 2N. The large inequalities in tail spotting may be due to the smaller number of individuals involved, still there were only about 100 more nose-spot animals than tail spot, so that differences due to numbers should not be great.

No matings have been made selecting for tail spot, but it is reasonable to suspect that results not differing greatly from those found in experiment 2N would be obtained. The same process of brother-sister mating has been carried out in both lines of family 2, and any matings of tail-spot animals have thus been made entirely at random. But since in the inbreeding process a constantly higher degree of homozygosis is being reached from generation to generation, and selection is taking place automatically, further selection from closely related individuals would not alter materially the type already present.

AREAS OF PIGMENTATION

Allen (*1*) has pointed out that animals have regularly pigmented areas. In most birds and mammals there are five of these centers on each side and a median one on the forehead. These patches are independent of one another and there may or may not be bilateral symmetry. The pigment areas are the coronal, or crown patch, aural or ear patch, nuchal or neck patch, scapula or shoulder patch, pleural or side patch, and sacral or rump patch. In most animals the rump patches remain fused dorsomedially and give the appearance when reduced of a single median patch at the base of the tail. The ear patch seems to be the most persistent, agreeing in this respect with the observation of MacCurdy and Castle (*2*), who say that in guinea pigs spots occur more frequently on the head than on any other part of the body.

These results conform closely with those obtained in studies of the stock of guinea pigs of the Bureau of Animal Industry. No definite investigation has been made of the regions of pigmentation, but observation of the animals clearly shows this regional division, and in many instances it is very definitely marked.

It also appears that the spots are independent of one another, for there is usually a noticeable lack of bilateral symmetry, and the pigmented areas even on the same side are not usually of the same color. The persistence of the head spots also seems to be in agreement, for often animals are found with no color on the body except a small patch on the ear or around the eye or sometimes a few colored hairs between the ears.

SUMMARY

In experiments with guinea pigs by members of the Bureau of Animal Industry, one family (family 2) used in the inbreeding experiments was found to produce a number of nose-spot and tail-spot animals.

This family became divided into two large lines in the eighth and ninth generations, one line producing more than 14 per cent of nose-spot young, the other a little more than 3 per cent.

The line producing a high percentage of nose spot produced a low percentage of tail spot—a little less than 3 per cent, while the other line produced nearly 10 per cent of tail spot.

Other inbred families produced slightly more than 1 per cent nose-spot and tail-spot young. In crosses between family 2 and four other inbred families slightly more than 3 per cent of nose spot was produced. Crosses of the high and low producing lines of family 2 with family 13 showed slightly more nose spots from the high line than from the low, and a slight increase in the F_2 and F_3 generations over the F_1.

A selection experiment, 2N, did not produce more nose-spot animals than the high-producing line of family 2. The amount of nose-spot ancestry had little effect on the number of nose-spot young, except that there was a lower percentage of nose spot when none of the parents, grandparents and great-grandparents had nose spot.

There is little or no relation between the nose-spot character and birth weight, size of litter, mortality, season of birth, sex, or litter rank in which such pigs were born. The difference in the amount of white on nose-spot animals and those without it can be accounted for by the spot itself.

Nose spot and tail spot appear to be inherited, but the inheritance can not be explained by simple Mendelian factors. Different types of matings produced all types of young in about the same proportions.

In family 2 and 2N there was an excess of red spots on the nose, tail, right shoulder, and left hip. This excess is in about the same ratio as red to black on the whole coat area of the animal, except that the nose shows a greater tendency to be red, if it is colored at all, than other portions of the animal.

The necessary factors for the tricolor pattern ($e^p e^p ss$) being present, the location of the spots themselves appears to be determined by local conditions in the developing skin. These conditions are very irregular in the guinea pig, but a slight heredity of the tendency of the colors to develop in certain spots exists in certain lines.

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THE EFFECT OF SUCCESSIVE GENERATIONS OF YEAST ON THE ALCOHOLIC FERMENTATION OF CIDER [1]

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INTRODUCTION

With the rapidly increasing demand for unfermented fruit juices during recent years much interest has been aroused in methods of manufacture. Although fruit juices are very attractive when first pressed they soon lose their attractiveness by yeast development, and consequently can not be handled or stored properly without being subjected to some process to check fermentation. Any treatment subsequent to pressing seems to injure more or less the delicate flavors and aromas of the juices. Filtering may improve the appearance but it does not improve the natural flavor. Chemical preservatives added in certain proportions may check fermentation but they often impart disagreeable flavors to the juices. Moreover, the use of such preservatives is of questionable propriety, and, as their presence is generally looked upon with suspicion by the public, their use is gradually decreasing. Pasteurization, although free from some of the objectionable features of the chemical preservatives, often gives a cooked taste and checks fermentation only so long as the treated juices are kept out of contact with the air. Refrigeration approaches perhaps most nearly the ideal of preserving the natural flavors of the juices, but it is an expensive process and unless the temperature is constantly kept at or near the freezing point of the juices fermentation starts readily and makes the product unfit for unfermented beverages. Since both pasteurization and refrigeration fail to produce unfermentable products, the objection that the juices so treated may be used for unlawful purposes remains unsolved. With the exception of the rather unsatisfactory use of chemical preservatives, there is yet no reliable method for the preparation of unfermentable fruit juices. In view of the rapidly growing demand for unfermented beverages it is evident that there is an urgent need for some practical method whereby clear, attractive, unfermentable fruit juices can be prepared with the least possible loss of natural flavors and aromas. There is still much to be learned about the fermenting organisms and their mineral food requirement. Careful research in this direction will undoubtedly bring to light much valuable information and may open the way to a successful solution of the unfermented-beverage problem. The work described in this paper is a preliminary study dealing with the application of some of the known basic factors in fermentation in an attempt to open the way to the development of some practical method of making unfermentable fruit juices without the use of chemical preservatives.

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Key No. Wash.-11

SCOPE OF THE INVESTIGATION AND METHODS OF PROCEDURE

It has frequently been observed that bacterial cultures develop very poorly or refuse to grow at all in bouillon media that have once served for the growth of these same bacteria. It seems that the by-products accumulated by the first generation make the media unsuitable for the growth of successive generations. This observation is not limited to bacteria but applies equally well to yeast. When alcoholic fermentations are checked by rising temperatures, which are normal in active fermentation, it is more difficult thereafter to resume the fermentation in the normal way. The reasons generally ascribed for this behavior are that the increased temperature resulting from very active fermentation kills many of the active yeast cells and that the by-products of these dead cells retard or prevent the maximum development of the new generation. It is this assumption perhaps which induced Boulard[2] to study the phenomenon with the end in view of using it methodically in the control of alcoholic fermentations of wines. By growing successive generations of yeast in wines he succeeded in rendering them totally unfermentable long before all the fermentable sugars were used up. According to his expression these wines were "immunized" or "vaccinated" against yeast and also against spoilage bacteria. Although the causes of this so-called immunization or vaccination have not been studied and are as yet insufficiently understood, it is presumed that the accumulated by-products of preceding generations of yeast are toxic to the extent that further yeast or bacterial development is altered or even prevented. It is also thought that successive generations of yeast soon exhaust certain essential food elements so completely that the medium is no longer capable of supporting yeast and bacterial growth. These two assumptions constituted the main object of this study. Cider pressed from cull apples of mixed varieties was used in the experiments.

The toxicity studies were carried out by placing definite amounts of this cider in Erlenmeyer flasks, inoculating them with a heavy suspension of yeast culture, and incubating them at 25° C. Active fermentation was checked at will by heating the inoculated cider at 45° C. for one hour. As this treatment killed many of the active yeast cells, reinoculation following each heating was necessary. The process was repeated until the yeast refused to develop. Three or four inoculations were usually sufficient to accomplish this end.

Because of the well-known fact that cider is generally very low in nitrogen and phosphorus, both of which are believed to be indispensable for the growth and reproduction of yeast, the study of the possible depletion of certain food elements by yeast growth was limited to quantitative and qualitative determinations of these two elements. Determinations of alcohol and acetic acid were also made periodically but only for the purpose of following the course of their formation and of ascertaining how low their percentages could be kept during the process.

All the quantitative chemical determinations were made according to the official methods of the Association of Official Agricultural

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[2] BOULARD. SUR UN PROCÉDÉ PERMETTANT D'ARRETER À VOLONTÉ LES FERMENTATIONS A N'IMPORTE QUEL MOMENT. Compt. Rend. Acad. Agr. France 12: 615-620. 1926.

Chemists.[3] The qualitative tests for nitrites were made with Trommsdorf's reagent, those for nitrates with diphenylamine reagent, those for ammonia with Nessler's reagent, and those for orthophosphates with ammonium molybdate reagent followed by reduction with tin metal according to the method of Spurway.[4]

EXPERIMENTAL DATA

TOXICITY AND ALCOHOL PRODUCTION OF SUCCESSIVE GENERATIONS OF YEAST AND EFFECT ON NITROGEN AND PHOSPHORUS CONTENT OF CIDER

Three 2-liter Erlenmeyer flasks were filled with 1,500 c. c. of freshly pressed cider and two of them were heated at 45° C. for one hour and then cooled to 25°. Since heating caused considerable precipitation in the cider, the precipitate was first allowed to settle and was then separated by decantation. The supernatant liquid and sediment were analyzed separately for nitrogen and phosphorus. As the concentration of sediment naturally varied in different decantations, the results of the analysis of the various sediments could not be expected to agree very closely, but the analysis was made only to determine the approximate proportion of these elements in the two separated parts. When the analysis was completed the sediment was rejected but the flasks with the supernatant liquid of the heated cider and that with the unheated fresh cider were inoculated with 2 per cent of a heavy suspension of *Saccharomyces valesiacus* (Osterwalder) at its maximum activity in sterilized cider. The inoculum was thoroughly mixed with the cider in the flasks and allowed to develop at 25° until the fermentation became active as shown by the appearance of foam bubbles on the surface of the liquid. The three flasks were then heated at 45° for one hour, the heating causing once more considerable precipitation which was allowed to settle before being separated by decantation. The analyses for nitrogen and phosphorus of the supernatant liquid and sediment were made separately as before. Reinoculations with consequent heatings and analyses were repeated three times, resulting in progressively smaller amounts of precipitated material and a considerable decrease in yeast development each time. A fourth inoculation was made in which the amount of inoculum was twice that which was used previously. The cider at that time was already clear and remained so even after 24 hours' incubation at 25°. Neither was there the slightest indication of any yeast development observable by macroscopic means during that time. The results of the various analyses obtained up to this stage are reported in Table 1.

The data indicate that cider is very low in nitrogen and phosphorus, that a large part of these elements was easily precipitated by a temperature of 45° C., and that most of that remaining was used up by the first generation of yeast. However, the removal of the last traces of ammonia nitrogen and orthophosphates proved to be extremely difficult as indicated by the results of the qualitative analyses. A positive test for these substances was secured as long as the experiment was in progress, but the nitrogen was never found in the nitrite or nitrate forms in any of the experiments.

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[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

[4] SPURWAY, C. H. TEST SOILS FOR WATER-SOLUBLE PHOSPHORUS. Mich. Agr. Expt. Sta. Quart. Bul. 9: 64-65. 1926.

TABLE 1.—*Analysis of cider and sediment in the first experiment at different stages of the treatment*

Treatment	Quantitative analysis in percentages						Qualitative analysis [a]			
	Supernatant liquid				Sediment		Supernatant liquid			
	C_2H_5OH by weight	Acidity in terms of CH_3COOH	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrites	Nitrates	Ammonia	Orthophosphates
Cider unheated before first inoculation:										
Fresh cider	0.00	0.40	0.0098	0.0028			−	−	+	+
First inoculation			.0		0.035		−	−	+	+
Second inoculation			.0	.0	.0056	0.0061	−	−	+	+
Third inoculation	.56	.44	.0		.0002		−	−	+	+
Cider heated before first inoculation:										
Fresh cider heated at 45° C. for 1 hour	.00	.39	.0056	.0013	.028	.0041	−	−	+	+
First inoculation			.0		.039		−	−	+	+
Second inoculation			.0	.0	.013	.0082	−	−	+	+
Third inoculation	.53	.44	.0	.0	.0024	.00	−	−	+	+

[a] + = positive test; − = negative test.

The effect of successive generations of yeast was shown by a cell development which was in inverse proportion to the number of generations. The third generation grew very poorly and the fourth failed to show any signs of growth for some time at least. By carefully checking the development of each generation as soon as a few foam bubbles appeared on the surface of the cider, it was possible to limit the formation of alcohol to as small amounts as 0.56 and 0.53 per cent. Although it is true that even the smaller amount is slightly in excess of the limits permitted by the law applying to unfermented beverages, probably by more carefully checking the development of the yeast this excess could be reduced sufficiently to comply with the requirements of the law.

The change in total acidity during the process was insignificant and the results in general were most gratifying in every respect up to this point.

At the end of 24 hours' incubation following the fourth inoculation plate counts for the number of living yeast cells in the treated ciders were made on plain agar supplemented with 10 per cent cider. This modified plain agar was used throughout this study because the yeast developed better on it than on the plain agar. The plate counts were repeated after 5 days' incubation and also after 10 days' incubation. The results together with those of the qualitative tests for ammonia nitrogen and orthophosphates are recorded in Table 2.

TABLE 2.—*The determination of active yeast cells, ammonia, and orthophosphates in cider at different stages of incubation after the fourth yeast inoculation in the first experiment*

Treatment	Living yeast cells per cubic centimeter [a]	Qualitative analysis [b]	
		Ammonia	Orthophosphates
24 hours after fourth inoculation	1,760,000	+	+
After 5 days' incubation at 25° C	950,000	+	+
After 10 days' incubation at 25° C	1,600,000	+	+

[a] Each figure in this column is the average of counts taken from 4 plates.
[b] The plus sign indicates positive tests.

In spite of the fact that there was no visible change in the cider during the first few days following the fourth inoculation the data show that the yeast cells seemed to remain alive. At least they did not die at any abnormal rate, nor did they develop to any noticeable extent. On the other hand, bacterial growth, probably resulting from air contamination, seemed to make some progress, for on the fifth day of incubation a very light film was plainly distinguishable on the surface of the cider. On the sixth day some activity, apparently caused by the production of gas, was noticeable, and on the seventh day it was quite evident that at least some of the yeast cells were active. The plate count on the tenth day bore out this evidence by giving a decided increase in numbers of yeast colonies over those of the fifth day. Evidently the cider was not permanently unfermentable nor was it totally immune to bacterial growth. It was thought that the generations of yeast had perhaps not been given sufficient time to develop the necessary amount of toxic substances and so it was decided to repeat the experiment in a somewhat modified way.

In the second experiment two 4-liter flasks were filled with 3.5 liters of freshly pressed cider and heated for one hour at 45° C. The coagulated material resulting from the heating was allowed to settle and was then separated by decantation. The supernatant liquid and sediment were analyzed separately for nitrogen and phosphorus as in the foregoing experiment. One of the flasks containing the supernatant liquid was inoculated with 2 per cent of a heavy suspension of the natural cider flora at its maximum activity in cider and the other with a heavy suspension of *Saccharomyces valesiacus* as in the first experiment. The procedure from this point on was similar to that already described except that the development of the yeast, and consequently the fermentation, was allowed to continue a longer time for each generation before being checked by heating.

TABLE 3.—*Analyses of cider and sediment in the second experiment at different stages of the treatment*

Treatment	Quantitative analysis in percentages						Quantitative analysis [a]			
	Supernatant liquid				Sediment		Supernatant liquid			
	C_2H_5OH in weight	Acidity in terms of CH_3COOH	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrites	Nitrates	Ammonia	Orthophosphates
Heated fresh cider	0.0	0.43	0.0014	0.0029	0.0196	......	−	−	+	+
Cider inoculated with natural yeast flora:										
First inoculation	.0	......	.0	......	.093	......	−	−	+	+
Second inoculation	......	......	.0	.0	.101	......	−	−	+	+
Third inoculation	1.6	.42	.0	......	.0	......	−	−	+	+
Cider inoculated with S. valesiacus:										
First inoculation	.0	......	.0	......	.106	......	−	−	+	+
Second inoculation	......	......	.0	.0	.098	0.0105	−	−	+	+
Third inoculation	1.6	.43	.0	......	.0	......	−	−	+	+

[a] + = positive test; − = negative test.

At the fourth reinoculation the flasks were incubated at 25° C. and plate counts for yeast were made 24 hours later. The cider was then bottled in pint bottles and sealed by means of metal caps. Two bottles taken from the cider inoculated with the natural flora and two from that inoculated with *Saccharomyces valesiacus* were pasteurized for 30 minutes at 80° C. and after cooling were placed in storage in the 25° incubator with the rest of the bottles. Two months later and again four months later agar plates were prepared for yeast counts of both the pasteurized and unpasteurized bottles, and at the same time determinations for alcohol, nitrites, nitrates, ammonia, and orthophosphates were made. The data recorded in Tables 3 and 4 show that the results of this experiment were quite similar to those of the first experiment.

TABLE 4.—*Determination of active yeast cells, ammonia, orthophosphates, and per cent of alcohol in cider at different periods of incubation after the fourth inoculation with yeast in the second experiment*

Treatment	Living yeast cells per cubic centimeters [a]	C_2H_5OH	Nitrites	Nitrates	Ammonia	Orthophosphates
Cider inoculated with natural yeast flora:						
24 hours after fourth inoculation	300,000	1.60	—	—	+	+
After 2 months' incubation at 25° C	9,850,000	1.90	—	—	+	+
After 4 months' incubation at 25° C	1,700,000	2.60	—	—	+	+
Cider inoculated with S. valesiacus:						
24 hours after fourth inoculation	930,000	1.60	—	—	+	+
After 2 months' incubation at 25° C	240,000	1.87	—	—	+	+
After 4 months' incubation at 25° C	25,000	2.70	—	—	+	+
Cider inoculated with natural yeast flora of cider and that inoculated with S. valesiacus, but Pasteurized 24 hours after fourth inoculation:						
24 hours after fourth inoculation	0	1.60	—	—	+	+
After 2 months' incubation at 25° C	0	1.60	—	—	+	+
After 4 months' incubation at 25° C	0	1.60	—	—	+	+

[a] Each figure in this column is the average of counts taken from 4 plates. duplicate plates being prepared from 2 bottles.

There was a natural increase in alcoholic content due to the extra time each generation of yeast was allowed for development. However, this greater development of the several generations of yeast did not have the effect that was hoped for, namely, that of making the cider permanently unfermentable. Only the pasteurized bottles proved to be inactive. In all the other bottles fermentation took place slowly but continuously during the four months the cider was kept in storage. The renewed activity of the yeast probably began about the second week of incubation as in the first experiment. Although fermentation progressed to a certain extent the conditions for growth were never favorable. This was especially true for *Saccharomyces valesiacus*, as proved by the rapidly decreasing number of living cells during the four months of incubation. The yeast of the natural flora of the cider, probably composed of more than one species, behaved somewhat differently. The percentage of inoculum at the fourth inoculation being the same for the two yeasts, the concentration of the living cells might be expected to be similar also. But this was not the case, for the plate count showed that the number of living yeast cells was much smaller in the cider inoculated with the natural flora than in that inoculated with *Saccharomyces valesiacus*. However, as the time of incubation advanced the concentration of living cells became greater in the former than in the latter, indicating that certain species of yeast are more resistant to the effect of their by-products than others. But, on the other hand, the amount of alcohol produced by the two kinds of yeast was approximately the same though the number of living cells varied widely. The reason for this may be explained by the fact that the members of the species *Saccharomyces valesiacus* which are known for their high alcohol production are probably more efficient alcohol producers than the members of mixed species generally found in cider. Furthermore, the number of the growing cells which appeared on the agar plates are not necessarily an indication that all these cells were active under the extremely unfavorable conditions of the cider. Many may have been inactive in the cider and the alcohol produced may have been the result of the activity of comparatively few cells in both cases.

It is interesting to note that the number of living cells in both kinds of yeast declined rapidly during the last two months of incubation. It may be that the increasing pressure resulting from continued CO_2 production in the sealed bottles was a contributing factor. Or possibly the mineral food shortage became more intense as the fermentation progressed. Either or both of these factors may reasonably be expected to exert some influence on the development of living cells. In order to ascertain to what extent they were responsible for the conditions in the bottled cider the following experiment was prepared:

Fourteen 250 c. c. cotton-plugged Erlenmeyer flasks were sterilized and after being cooled 12 of them received 100 c. c. each of the cider that had been pasteurized at the fourth inoculation of the previous experiment and was known to be free of living yeast cells. The two remaining flasks received 100 c. c. each of sterilized freshly pressed cider. The flasks were arranged in 2 series of 7, each series containing 1 flask of the sterilized fresh cider and 6 of pasteurized cider that had been treated by successive generations of yeast. One series was inoculated with 1 c. c. quantities of cider from the sealed bottles treated with the natural yeast flora of cider in the previous experiment and the other series was inoculated with 1 c. c. quantities of cider from the sealed bottles treated with *Saccharomyces valesiacus* in the previous experiment. All this was done aseptically to eliminate possible errors from contaminations. The cotton plug in each flask was then replaced by a sterilized rubber stopper through which one end of a glass tube bent at right angles was passed. The other end was connected to a similar tube inserted in N/1 KOH in an absorption tower for the purpose of collecting the CO_2 produced. The two series were further treated as follows:

The flask containing the sterilized fresh cider and the first flask containing the pasteurized treated cider in each series were not treated and served as controls.

The second flask of pasteurized treated cider in each series received 0.1 per cent peptone.

The third flask of pasteurized treated cider in each series received 0.05 per cent of ammonium nitrate.

The fourth flask of pasteurized treated cider in each series received 0.1 per cent secondary potassium phosphate.

The fifth flask of pasteurized treated cider in each series received 0.05 per cent ammonium nitrate and 0.1 per cent secondary potassium phosphate.

The sixth flask of pasteurized treated cider in each series received 0.1 per cent peptone in addition to 0.05 per cent ammonium nitrate and 0.1 per cent secondary potassium phosphate.

The added materials were thoroughly mixed with the cider in the flasks and the two series, properly arranged and connected, were incubated at 25° C. for two weeks. At the end of the incubation period each flask was thoroughly shaken to expel the gases absorbed in the cider and the CO_2 collected in the absorption towers was determined by double titration, thymol blue and brom phenol blue being used as indicators. The data obtained are given in Table 5.

TABLE 5.—*The effect of peptone and mineral food elements on the activity of yeast in cider rendered temporarily unfermentable by successive generations of yeast*

Treatment	Per cent of alcohol and milligram of CO_2 in 100 c. c. after 2 weeks' incubation at 25° C.			
	Inoculated with natural flora from treated cider		Inoculated with S. valesiacus from treated cider	
	CO_2	C_2H_5OH	CO_2	C_2H_5OH
Pasteurized treated cider	57	----------	101	----------
Freshly pressed sterilized cider	5,394	5.61	5,614	5.94
Pasteurized treated cider+0.1 per cent peptone	2,824	5.53	2,270	5.40
Pasteurized treated cider+0.05 per cent NH_4NO_3	2,626	----------	1,408	----------
Pasteurized treated cider+0.1 per cent K_2HPO_4	272	----------	365	----------
Pasteurized treated cider+0.05 per cent NH_4NO_3+0.1 per cent K_2HPO_4	2,930	4.96	2,345	5.29
Pasteurized treated cider+0.05 per cent NH_4NO_3+0.1 per cent K_2HPO_4+0.1 per cent peptone	3,066	5.61	3,014	5.37

The active fermentation in the sterilized freshly pressed cider offers conclusive evidence that both kinds of yeast resumed their normal activity when exposed to the proper media. The data also indicate that the pressure existing in the sealed bottles as a result of CO_2 production had little or nothing to do with the rapidly decreasing number of living cells in the sealed bottles during the last two months of incubation. The addition of phosphates alone did not increase the activity of either kind of yeast, but the addition of ammonium nitrate caused a slight increase, showing that nitrogen was a limiting factor. When both ammonium nitrate and secondary potassium phosphate were added there was a decided increase in CO_2 production, indicating that one of the causes responsible for the limited activity of the yeast in the sealed bottles was lack of mineral food. The reason that more CO_2 was produced by the addition of peptone than by the addition of ammonium nitrate was perhaps that the organic form of nitrogen was better assimilated and more suitable for yeast growth than the inorganic form or that traces of phosphates were present in the peptone. The presence of traces of phosphates was indicated by a positive test for orthophosphates.

If the results of this experiment alone are considered it might be assumed that the extremely limited action of yeast in cider in which several generations had developed was chiefly due to lack of food. This assumption is supported by the fact that the addition of suitable forms of nitrogen and phosphorus caused a spontaneous and vigorous resumption of fermenting activity which resulted in a yield of alcohol approaching the normal amount in cider. But opposed to this assumption is the fact that the qualitative test for ammonia and orthophosphates was, as far as could be observed, identical at the beginning and at the end of the incubation period, while the number of living cells seemed to increase at the beginning but showed a rapid decrease at the end of the incubation period. Granting that the food supply was constant throughout the incubation period, it seems that the number of living cells should also have been approximately the same during that period if the food supply was the only limiting factor. It would therefore appear that other causes were partly responsible. The accumulation of toxic products, generally assumed to be derived from the by-products of metabolism or from disease conditions, but as yet poorly understood because of their complexity and the lack of adequate methods, was perhaps a contributing cause. It is true that the effect, if there was any, was ultimately of minor consequence, as is shown by the final high yield of alcohol, but whatever effect there was may have been counteracted totally by enzymic autofermentation. These factors will be considered more fully in a succeeding experiment.

Two important observations not shown by the data presented, but valuable from the standpoint of manufacture, were made in these experiments and should not be overlooked. One is that the heatings required in the process caused complete coagulation of the material in suspension, resulting in a clear and attractive cider without the usual slow and tedious operation of filtering. The other is that as far as could be ascertained by taste the heatings at this low temperature did not affect to any perceptible degree the natural flavor of the cider. The first is very important from the standpoint of cost of manufacture and the second is valuable from the standpoint of quality of the product.

EFFECT OF THE FILTRATE OF THE TREATED CIDER ON ALCOHOL PRODUCTION AND ON THE DEVELOPMENT OF YEAST

Since yeast enzymes are able to ferment the same sugars that the living cells secreting them ferment, it is possible that some of the alcohol in the stored cider was caused by autofermentation of zymase freed by the disintegration of dead yeast cells. As the temperature used in heating the cider for the purpose of checking the growth of the several generations of yeast was too low to inactivate the enzymes, many of them might be liberated at each heating by maceration, thus giving them an unusual opportunity to exert considerable influence on the fermentation. The extent of this influence is difficult to determine correctly because these free enzymes are not easily obtained in a pure state. The filtering membranes of the porous candles generally used for this purpose may or may not permit their free passage, depending on the electrical charges of the filter. However, the pressure commonly used on these filters counteracts the effect of the opposite electrical charge of the filtering membrane to a certain extent and forces some of the enzymes through the openings irrespective of electrical charges. Thus approximate results may be obtained by the use of the porous candle. In this experiment a portion of each of the two differently treated ciders used in the second experiment was filtered through a sterilized N. Berkefeld candle. Duplicate 100 c. c. quantities of each filtrate were mixed with 100 c. c. of Buchanan solution previously sterilized in 250 c. c. Erlenmeyer flasks. The Buchanan solution was added to provide mineral food substances which might be lacking in the filtrates. Special emphasis was given to soluble phosphates, as this substance seems to be necessary and is known to greatly accelerate the autofermentation of enzymes. According to Harden [5] autofermentation in media poor in phosphates may be increased from 10 to 150 per cent by the addition of suitable forms of phosphates. After thoroughly mixing the contents in the flasks, each flask was connected to an absorption tower in the manner described in the previous experiment and was incubated at 25° C. for two weeks. At the end of the incubation period the flasks were shaken vigorously to liberate any gases that might be absorbed in the solution and force them into the absorption towers to be taken up by the KOH solution. Determinations for CO_2 were made by the method previously used.

The amount of CO_2 formed was found to be so small as to be insignificant, indicating that autofermentation by enzymes was absent in the filtrates and supporting the assumption that the alcohol present in the treated ciders was probably the result of the usual activity of living yeast. As pointed out before, even this activity was very limited, as only about 1 per cent of alcohol by weight was formed during four months of incubation at the optimum temperature for yeast growth. Why the amounts of alcohol were approximately the same in both of the treated ciders irrespective of the wide variation in numbers of living yeast cells between that inoculated with the natural yeast flora and that inoculated with *Saccharomyces valesiacus* is not clear. It has many times been observed that certain individual cells of a given species of microorganism are able to perform their activities apparently unaffected in culture media which are toxic and prove to be decidedly destructive to the large majority of the cells. Likewise, certain cells of a given species have often been known to acquire a resistance to disease conditions, such as bacteriophage, to the point that they are able to grow and reproduce normally in the infected media while the majority of the cells are dissolved by the bacteriophage principle. Perhaps this same principle of adaptation or resistance is applicable to media with limited amounts of food supply. Thus it may be supposed that the majority of the yeast cells in the treated cider were unable to absorb sufficient food for growth and reproduction and finally died, while a few of the more resistant cells were able to adjust themselves to these unfavorable conditions and performed their normal activity successfully. Consequently, many of the yeast cells which were capable of growth and reproduction when plated out on favorable media were unable to perform their normal function of converting sugars into alcohol when exposed to the existing unfavorable conditions in the treated ciders. The result naturally followed that the alcohol formed was the product of the activity of comparatively few yeast cells. The fact that by far the larger part of the alcohol was produced during the last two months of incubation when the largest reduction of living cells was in evidence, seems to lend support to this supposition. That the limited activity of the yeast during the incubation period was mainly due to the limited food supply was demonstrated by the extent of the renewed activity resulting from the addition of suitable food substances and also by the final normal yield of alcohol. But, nevertheless, there is a possibility that accumulated toxic products or bacteriophage had some effect on the yeast development.

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[5] HARDEN, A. ALCOHOLIC FERMENTATION. Ed. 3, p. 57-58. London, New York, [etc.], 1923.

The effect of the suspected toxic products of the treated cider on yeast development was determined by an experiment in which 10 per cent of the filtrates of the treated ciders was added to the decanted supernatant liquid of fresh cider heated at 45° C. for one hour. The filtrates were obtained as in the previous experiment and were added at the first inoculation together with the usual 2 per cent of inoculum. The filtrate obtained from the cider treated with the natural flora of yeast was added to the fresh cider inoculated with the natural flora of yeast, while the one obtained from the cider treated with *Saccharomyces valesiacus* was added to the fresh cider inoculated with *S. valesiacus.* Following this treatment the experiment was conducted exactly like the second experiment, but while it was in progress careful observations were made of the behavior of the fermentation and of the growth of yeast. The entire behavior proved to be similar in all respects to that of the second experiment and because of this similarity the data are not given. The results showed that the addition of 10 per cent of the filtrates did not have any retarding effect on the development of yeast.

Determinations for the bacteriophage principle were made by filtering through sterilized N. Berkefeld candles several samples of cider of the first and second experiment after the third and fourth inoculations. Quantities of 1 c. c. of these filtrates were added to bouillon and plain agar tubes containing 8 c. c. of the respective media. These were seeded with 1 c. c. of a heavy suspension of *Saccharomyces valesiacus* and the agar tubes were used to pour plates. The plates and the bouillon tubes were incubated at 25° C. for a week and were examined carefully at short intervals for any dissolving action of bacteriophage. The experiment was repeated several times, but at no time could any lytic or solvent action be observed either in the plates or in the bouillon tubes, thus indicating that bacteriophage was not present in the cider and consequently had no effect on the yeast development.

DISCUSSION

The results of the preceding experiments show the possibility of rendering cider temporarily unfermentable by growing successive generations of yeast and also the probability that the alcohol content produced during the process can be kept within the limits prescribed by the law for unfermented fruit juices. It was found that the factors chiefly responsible for the unfermentable condition of the cider are not the accumulated toxic products of preceding generations or the effects of disease conditions of the yeast itself, but the lack of nitrogen and phosphorus. The reason that the fermentation in the treated cider resumed a limited activity under optimum conditions for yeast growth after a week of apparent inactivity is probably due to the fact that the small traces of mineral food present in the cider were sufficient to support the growth and reproduction of a few yeast cells. That these traces of nitrogen and phosphorus did not seem to disappear with the subsequent limited yeast activity may be explained by the supposition that through the process of hydrolysis of the dead cells new supplies of available forms of these elements sufficient to maintain the life processes of a comparatively small number of cells were constantly being provided. Complete removal of nitrogen and phosphorus or even of phosphorus alone would very likely result in a permanently unfermentable product. It is generally agreed that the development of yeast is impossible in the total absence of phosphorus, and Harden[6] states that fermentation by zymase should not occur in the total absence of phosphates.

In this study only partial success in removing either the nitrogen or the phosphorus was obtained, but it should be remembered that the work was preliminary and that further research may accomplish much. The results obtained seem to indicate that the best hope for success in this process lies in the total removal of phosphorus, because it prevents both fermentation and autofermentation. If this could be accomplished successfully the process would offer many obvious advantages. Manufacture would be simplified by eliminating the slow and tedious operations of filtering, but would nevertheless give an attractive, clear beverage possessing the natural flavor of the unprocessed freshly pressed cider. The elimination of the filtering operation would greatly reduce the cost of the initial capital outlay for machinery, thereby making possible the building of small plants at centrally located points and thus vastly diminishing the cost of transportation of the bulky raw material. In this way both manufacturer and producer would realize better returns, and a large per centage of the cull fruit now going to waste could be utilized in the form of a healthful refreshing beverage.

SUMMARY

A preliminary study was made of the effect of successive generations of yeast on the alcoholic fermentation and the beverage quality of cider.

The results showed that the nitrogen and phosphorus content of cider was low and that all but traces of each were readily removed by coagulation at a temperature of 45° C. and the growth of two or three generations of yeast.

Qualitative analyses indicated that nitrogen was at no time present in the nitrate or nitrite form, but was always present in small amounts in the form of ammonia. A positive qualitative test was at all times obtained for orthophosphates.

Clear cider, containing 0.53 per cent of alcohol and possessing the natural flavor of unprocessed, freshly pressed cider, was obtained in a temporarily unfermentable form by the growth of three generations of yeast.

The chief factors responsible for the temporary unfermentable condition of the cider were found to be the lack of nitrogen and phosphorus and not the effect of accumulated toxic products or of disease conditions of the yeast.

It was observed that the temporarily unfermentable cider was subject to a limited amount of fermentation after seven days of incubation at the optimum temperature for yeast and that this was probably due to the presence of traces of available nitrogen and phosphorus which were sufficient to maintain the life processes of a small number of yeast cells.

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[6] HARDEN, A. Op. cit.

EFFECT OF VARIATION OF POTASSIUM AND CHLORINE IN A WHEAT RATION[1]

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INTRODUCTION

In a paper by Olson and St. John (*7*),[2] it was suggested that the addition of sodium to a ration based upon wheat was of greater benefit than the addition of potassium, and that the use of sodium salts in place of potassium salts in experiments previously reported by other investigators might have entirely altered the results obtained. Following this work a study was made of the effect of varying the amount of potassium in a ration based upon wheat. A study of the effect of the variation of chlorine in this same basal ration was also made. The white rat was the experimental animal used in all of this work. The present paper reports these studies and is the second progress report on the project dealing with the nutritive value of wheat.

REVIEW OF LITERATURE

Miller (*4*), using only 0.037 per cent of potassium in a synthetic diet, found that poor growth resulted. He believes that the minimum potassium requirement is between 0.055 and 0.144 per cent in such a ration. He states that the growth of rats can be greatly retarded by reducing the amount of potassium below approximately 1 per cent. He does not believe that sodium can replace potassium. A ration with a ratio of potassium to sodium of 14:1 is said to have no deleterious effect on the growth of young rats. Miller (*5*) found that the introduction of potassium salts into a synthetic diet caused immediate but temporary increase of sodium and chlorine excretion in the urine. He believes that the potassium requirements for animal development are abundantly satisfied in the ordinary ration.

Richards, Godden, and Husband (*10*) feel that the assumption that any ordinary ration will contain sufficient sodium and potassium is probably right. The addition of potassium citrate to a ration of cereal grain containing maize, oatmeal, barley, and blood meal fed to growing pigs lead to a decreased assimilation of nitrogen, phosphorus, and calcium. They feel that any adverse effect of large amounts of potassium is probably due to the depressing influence on the metabolism of these three elements and not to the impoverishment of the organism in sodium and chlorine.

Osborne and Mendel (*8*) report growth of rats on a synthetic ration containing 0.03 per cent potassium, although when both sodium and potassium were low, growth ceased. Their control diet contained 0.83 per cent of potassium. They also report completion of

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[2] Reference is made by number (italic) to "Literature cited," p. 63.

growth on less than 0.04 per cent of chlorine. They quote Rosemann (*11*) as demonstrating that a diet deficient in chlorides leads to an insignificant reduction in the total chlorine content of the body. They suggest that the result is attributable to a husbanding of this element. Richards, Godden, and Husband (*10*) believe that the theory of chlorine impoverishment caused by high potassium intake

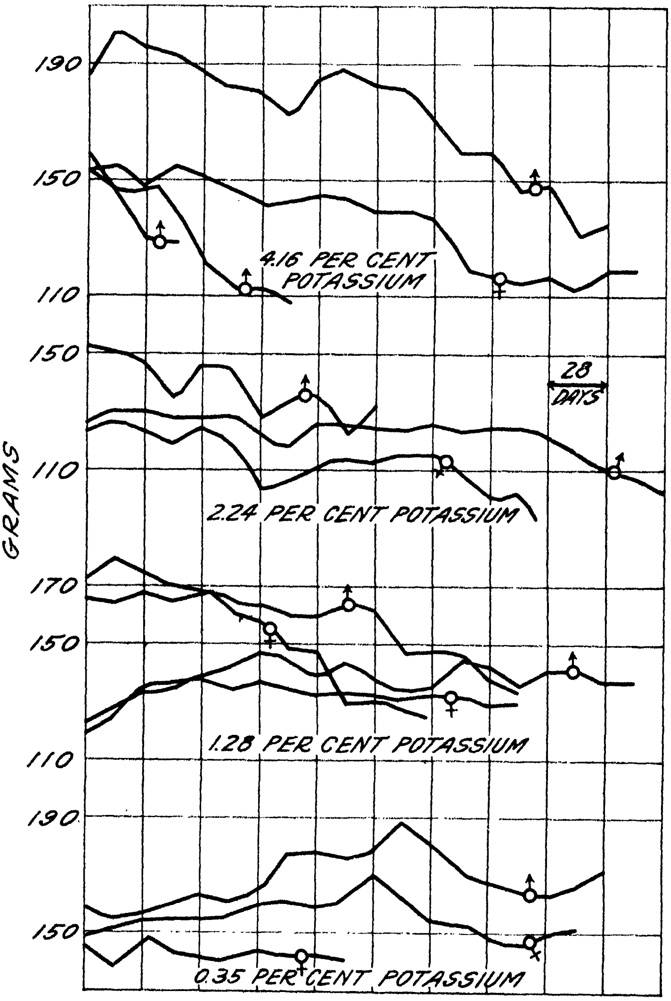

Fig. 1.—Growth curves, showing that rations containing added potassium do not maintain partially grown rats

is quite untenable and then feel that their results show the rapidity with which the animal body can adjust itself to sudden changes in the diet. The same authors (*9*) believe that their results indicate that their animals assimilated more of the chlorine than of the sodium when salt was added to the ration. Mitchell and Carman (*6*) improved growth by adding sodium chloride to a corn ration.

EXPERIMENTAL DATA

POTASSIUM

The basal ration used in the experiments on potassium was the same as that described by Olson and St. John (7) and consisted of 100 gm. of wheat, 10 gm. of gluten, 5 gm. of prepared butterfat, and 0.5 gm. of calcium chloride. This ration contained 0.32 per cent

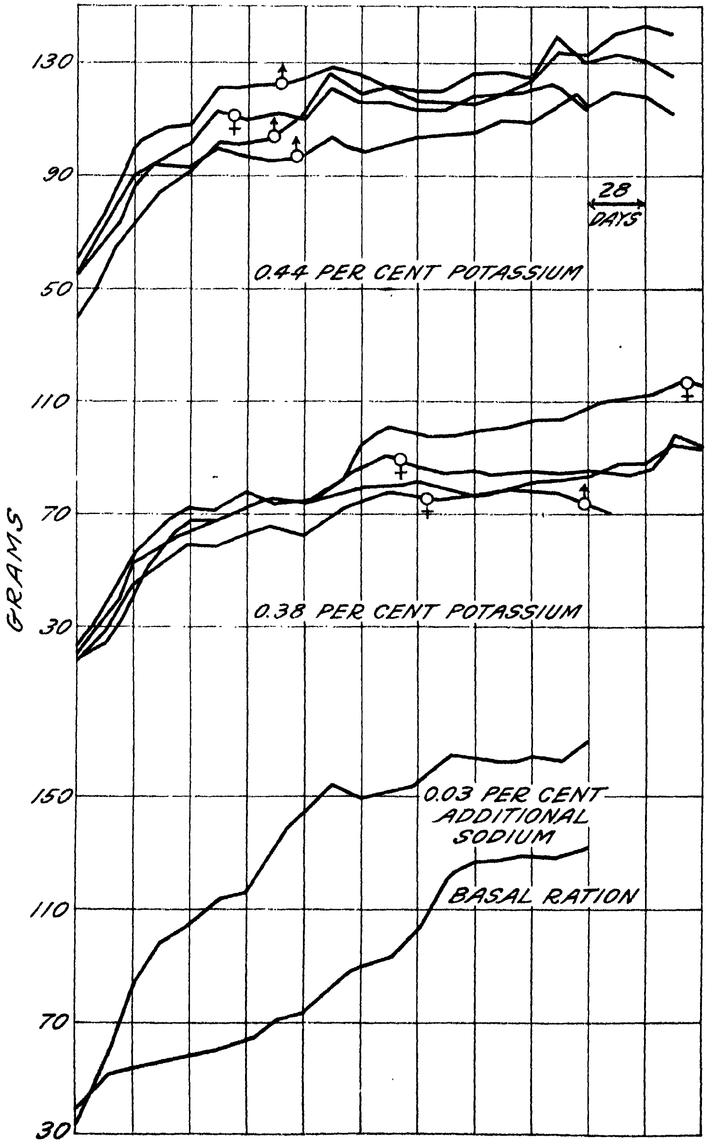

FIG. 2.—Curves showing the effect of potassium on the growth of young rats

potassium. Different amounts of potassium bicarbonate were added to the basal ration to form rations containing from 0.35 to 4.16 per cent of potassium.

Stock rats of fair size in good condition and growing rapidly were placed on these rations to determine the effect of the added potassium upon maintenance. The growth curves for the animals receiving the minimum and the largest amounts of added potassium are shown in Figure 1. The ration containing the minimum of potassium appears to be about a maintenance ration for animals of this age. Reference to the growth curve for the basal ration shown in Figure 2, copied from the paper by Olson and St. John (7), indicates that the basal ration carried the animals slowly to about 130 gm. and then maintained them, while with sodium added growth was very much improved. On rations containing amounts of potassium from 1.28 to 4.16 per cent the animals declined in weight. The average amount of food consumed per animal per week was 69.5 gm. on the ration containing the smallest amount of potassium and 59.0, 64.4, and 75.7 gm. on the three containing the larger amounts of potassium. The ratio of potassium to sodium was, respectively, 1.52:1, 5.56:1, 9.73:1, 18.1:1 in these four rations.

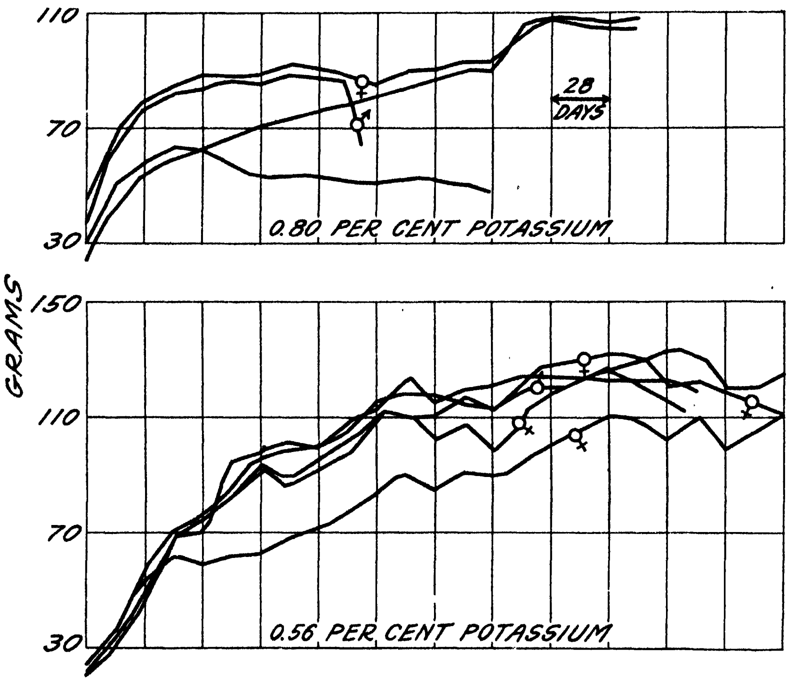

FIG. 3.—Curves showing the effect of potassium on the growth of young rats

Figures 2 and 3 present the growth curves for rats on four rations containing from 0.38 to 0.80 per cent of potassium. Variation of this element in the ration when added in the form of potassium bicarbonate seems to have comparatively little effect on the growth of young rats. Increasing the amount did not effect the improvement in growth which was effected by the addition of increasing amounts of sodium in the form of sodium bicarbonate as shown by Olson and St. John (7). The ratio of potassium to sodium in these rations varied from 1.65:1 to 3.47:1. The amount of feed consumed per week by these animals increased with increasing amounts of potassium from about 32 gm. to about 40 gm. per rat. There was no reproduction among the rats on any of these rations and no pregnancies were noted.

CHLORINE

The same basal ration was used for the study of chlorine as for the study of sodium and potassium. To the wheat, gluten, and butterfat were added various combinations of calcium chloride, calcium carbonate, sodium bicarbonate, and sodium chloride, the proportions being arranged to keep the percentage of calcium constant and the sodium constant (except in the ration containing the largest amount of chlorine), while the chlorine varied from the 0.05 per cent in the basal ration to a maximum of 1.14 per cent. The mineral additions to the rations are shown in Table 1.

TABLE 1.—*Mineral additions made to vary the chlorine content of the ration*

Chemical added	Added to basal ration for —					
	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5	Ration 6
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Concentrated HCl (c. c.)						2.00
$CaCl_2$		0.125	0.250	0.500	0.50	.50
$CaCO_3$	0.45	.338	.225			
$NaHCO_3$	.572	.572	.572	.572		
NaCl					3.16	.40
Cl (per cent)	.05	.12	.19	.32	1.14	1.14

Figures 4 and 5 present the growth curves for rats on rations containing four different amounts of chlorine. The growth response on all of the rations appears to be about the same irrespective of the amount of chlorine present. The ration used by Olson and St. John (7) contained an intermediate amount of chlorine (ration D, 0.32 per cent Cl) and the response was here also about the same as in this work when an equal amount of sodium was used. The basal ration with only 0.05 per cent of chlorine produced as good growth as where large amounts of chlorine were used. The maximum amount of chlorine used did not appear to have a detrimental effect on growth, as proved to be the case when large amounts of sodium and potassium in the form of bicarbonate were employed. It will be noted from Figures 4 and 5 that reproduction was secured in several cases. The amount of feed consumed per rat per week decreased to some extent as the amount of chlorine in the ration increased.

It is interesting to note that in the ration containing the largest amount of chlorine a part of this element was added in the form of sodium chloride, so that the percentage of sodium was increased in this one ration and was present to the extent of 1.05 per cent, which amount had proved somewhat detrimental when added in the form of sodium bicarbonate. This suggests that the form in which an element occurs in a diet may influence its effect on growth. This point should be studied further. Another ration in which hydrochloric acid was used to bring the chlorine content up to 1.14 per cent gave very good growth and very satisfactory reproduction.

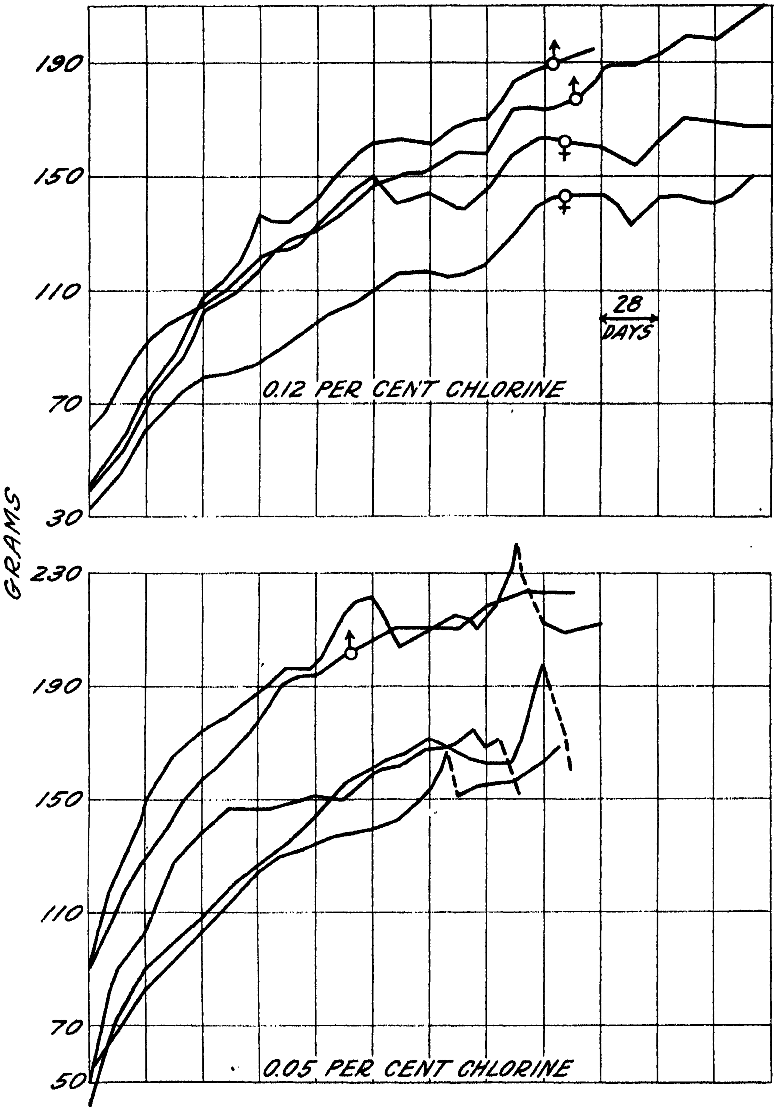

FIG. 4.—Curves showing effect of chlorine on the growth of young rats

DISCUSSION

The value of the ratio of potassium to sodium has been emphasized by other writers. In the work of the present writer this ratio has been varied from 0.12:1 to 18.1:1. With the wheat ration described above, the best results were obtained when this ratio was 0.6:1, the percentage of potassium and sodium being 0.32 and 0.53, respectively. With a ration of purified food materials containing casein, starch, lard, yeast, cod-liver oil, and an inorganic salt mixture, the writer (*12*) obtained the best results when the ratio was 1.76:1; a ratio of 2.76:1 gave very much poorer results. In all of this work the best results were obtained when sodium was present to the extent of about 0.5 per cent of the ration. From this and all of the work with which the author is acquainted it appears that there is not yet sufficient evidence to show that the ratio of potassium to sodium is in itself more important than the actual quantity of these elements in the ration. On the contrary, the evidence seems to support the view that the quantity present is the important factor and that the ratio

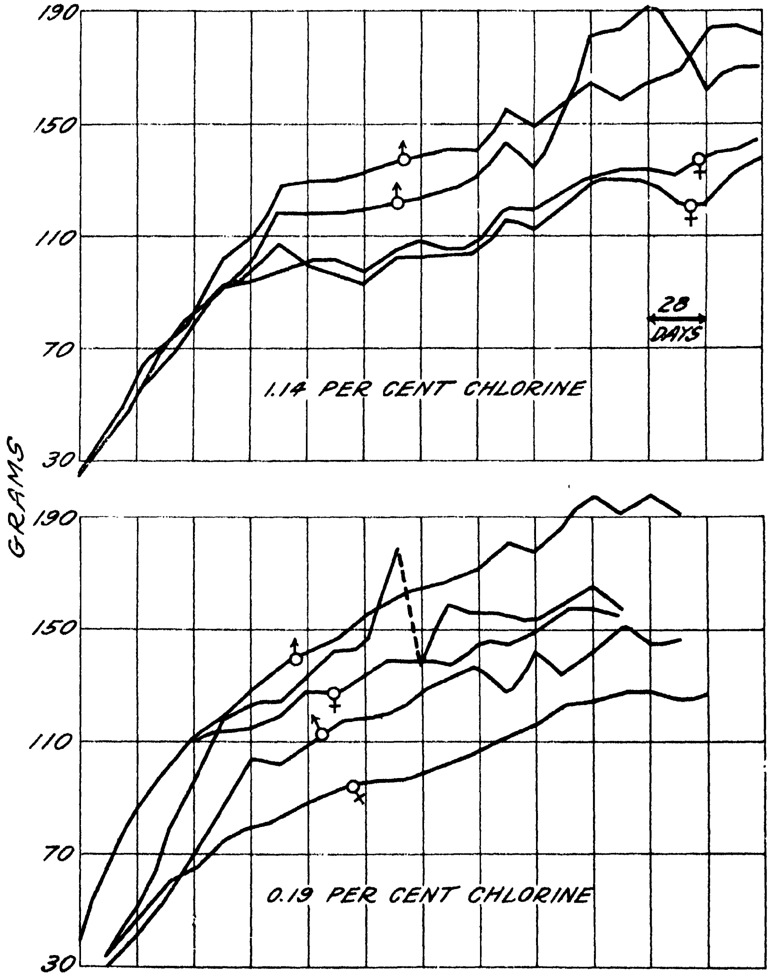

FIG. 5.—Curves showing the effect of chlorine on the growth of young rats

may be comparatively wide. What the ratio will be when the optimum for both of these elements has been finally determined is still a question.

The addition of potassium in the form of potassium bicarbonate to a ration based on wheat had very little beneficial effect on the growth of young rats. Neither was the addition of this element to the ration accompanied by reproduction. The results are in contrast to those obtained with sodium which had previously been found beneficial to growth and reproduction. This work emphasizes the view that sodium and potassium are not interchangeable, and it is apparent that potassium will not replace sodium in the ration used. Potassium when present to the extent of over 1 per cent of the ration proved detrimental to animals which had attained a weight of approximately 150 gm.

Although the experimental animals were not kept on screens, as were those used by the writer in a previous experiment (*12*), this fact would have no bearing on the results obtained in view of the work of Kennedy and Palmer (*2*), who state that it does not seem probable that the feces can add anything to the protein, inorganic salts, or energy content of the ration. They appear to feel confident that the feces do not supplement the ration by adding any one of the recognized food factors, and postulate a new vitamin. Such a vitaminlike substance would undoubtedly not be deficient in the type of ration reported upon here, so that the practice of coprophagy would not have a bearing upon these results.

The amount of feed used per gram of gain by the rats receiving the potassium bicarbonate was found to be somewhat greater than when potassium in this form was not added. Increasing the quantity of potassium in the ration appears to increase the food requirement per unit of gain. But Olson and St. John (*7*) have shown that an increase in the percentage of sodium in this ration effected a decided economy in the use of the feed, since the feed requirement per gram gain was decidedly less where larger proportions of sodium were incorporated in the feed. The effect of the potassium in decreasing the feed economy may be explained by the results of Richards, Godden, and Husband (*10*), who found that the addition of potassium to a ration of cereal grain led to a decreased assimilation of nitrogen, phosphorus, and calcium. The results reported by Olson and St. John (*7*) do not, however, agree with the assumption indorsed by these investigators that any ordinary ration will contain sufficient sodium and potassium, since it was found that sodium very materially improved the ration used.

It has been noted that there was no reproduction among the rats on the potassium rations while reproduction occurred among those on sodium rations, as previously reported. The work of Jacques Loeb and his coworkers may suggest a reason for this variation between sodium and potassium. They found that when the fertilized eggs of *Fundulus heteroclitus* were placed in a solution of either sodium chloride or potassium chloride a poisonous action was exerted by these salts. A solution containing both salts was less poisonous than a solution of either one alone, the relative toxicity varying with a variation in the relative concentration. According to Loeb and Wasteneys (*3*), it requires a much larger concentration of sodium chloride to exert a poisonous action than of potassium chloride.

Bechhold (*1, p. 378*), in discussing the work of other authors along this line, states that the potassium ions are especially poisonous because they change the state of turgescence of the organ colloids. If we assume that potassium and sodium salts have a similar action on the fertilized ovum of the rat, we have an explanation of the fact that reproduction was better among the rats on the rations containing added sodium than among those on rations containing added potassium. The addition of small quantities of potassium to the ration may have raised the concentration sufficiently to be toxic. The comparatively high concentration of potassium in the synthetic ration used by the present writer (*12*) may also have been a factor in the failure to secure reproduction among the rats on those rations.

SUMMARY

The results obtained by varying potassium and chlorine in a ration based upon wheat are reported. When rats weighing approximately 150 gm. were fed this ration with potassium bicarbonate added in quantity sufficient to incorporate more than 1 per cent of potassium the rats lost weight. Young animals receiving rations containing 0.4 to 0.8 per cent of potassium grew at about the same rate as those that received the basal ration. None of the animals receiving rations containing added potassium for any length of time produced young. Comparing these results with those previously reported, it appears that potassium will not replace sodium in a ration of this type.

The addition of chlorine to the wheat ration used did not effect an improvement in growth. Reproduction occurred in some instances among rats on this ration, although there is little indication that it varied with the quantity of chlorine present.

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THE WATER RELATIONS OF YAKIMA VALLEY SOIL[1]

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INTRODUCTION

There are strong economic reasons why in the Yakima Valley, Wash., as in many other irrigated sections, the most efficient use should be made of the available supply of irrigation water. The area of land available for irrigation is somewhat larger than can be irrigated adequately with the present water supply, and the local topography makes it necessary to lift some of the water to certain areas of the irrigable land.

The quantity of dissolved salts in the water supply of the Yakima Valley is less than 100 parts per million, and the proportion of alkaline-earth bases (calcium and magnesium) to alkaline bases (sodium and potassium) is high. There are a few areas in the valley where the subsoil is saturated with water and where the salt content of the soil solution is so high as to be troublesome. These water-logged or salty areas, which need drainage, are mostly confined to the lower lands. The higher bench lands generally have good subsoil drainage and appear to be in little danger of impaired productivity from the accumulation of subsoil water or salt.

The immediate practical problem of the irrigator in the Yakima Valley is how to make the most efficient use of the irrigation water that reaches his farm. This involves chiefly the proper leveling of the land to obtain uniform distribution of the water and the avoidance of the waste of water either by surface run-off or by percolation below the root zone. The losses from surface run-off can readily be seen, but those from percolation are not visible and can be detected only by subsoil exploration, which requires much labor and the use of equipment for determining soil moisture.

The chief purpose of the investigations reported in this paper was to bring together some of the simpler facts concerning the water-holding capacity of the type of soil that occurs on the higher lands of the Yakima Valley. Such information should be useful not only to the irrigators of that region but also to those of other regions. Irrigators generally need more precise information about the water relations of the soil. The soil acts as a reservoir in which to store water for the use of crop plants. The effective capacity of this reservoir is limited by the quantity of water the soil can hold against the force of gravity, by the quantity of water it can withhold from absorption by the plant roots, and by the depth of the root zone.

By a series of investigations such as here reported it is possible to estimate the quantity of water that can be stored in each successive

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layer of the root zone of an irrigated soil and the quantity that is withheld by the soil from plant absorption. The actual depth of the root zone is not the same for different soils or different crops. It is often limited by the depth to which irrigation water penetrates, though in some situations it is limited by the occurrence of a saturated zone in the subsoil. When the irrigator knows approximately how much water can be stored in the soil for a given depth of root zone he can estimate the quantity of water that will be required to penetrate to the desired depth.

These investigations were conducted at the irrigation branch station of the Washington State Agricultural Experiment Station,[2] near Prosser, Wash., on one of the areas to which water must be lifted by pumping. The present annual allotment of water for this area is 3 acre-feet per acre. This water is distributed to the fields by the "corrugation" method of irrigation, which is commonly used throughout the Yakima Valley. The water is conducted across the field in small furrows, and where the surface slope is slight or the soil is highly permeable there is some danger that the irrigation water may penetrate below the root zone at the upper end of the field before the lower end of the field has been irrigated sufficiently.

THE WATER RELATIONS OF THE SOIL

The soil of the Prosser experiment farm is classed as a fine sandy loam and is known locally as volcanic ash. It is of a type that is common throughout the Yakima Valley and the Columbia Basin. It contains very little clay and takes water readily. The only impediment to the rapid percolation of water through the root zone is the occurrence in spots of 1 to 2 inch layers of very fine silt having a compact and laminated structure. These layers may occur at any depth in the root zone or below it. When very compact, as they sometimes are, they retard the percolation of water temporarily. Their occurrence is one of the causes for the differences in moisture content found in duplicate samples taken from the same plot. This fine silt holds more water than the coarser soil and thus causes aberrant results for moisture determinations.

The general moisture relations of the soil may be indicated by reference to the so-called moisture equivalent. This term may be defined as the quantity of water, expressed as a percentage of the dry weight of the soil, that is held by the soil against a centrifugal force one thousand times the force of gravity.[3] In order to afford a basis of comparison to the other soils of which the moisture equivalent is known, samples of the Prosser soil were submitted to J. W. McLane, of the Bureau of Plant Industry, for the determination of their moisture equivalents. These samples were taken to represent each foot in depth to 6 feet. Twelve samples were taken for each section, and these were thoroughly mixed to make a composite for each foot. The results of the moisture-equivalent determinations are shown in Table 1. In converting the figures for percentage of moisture into those for inches of water it is assumed that the dry soil as it occurs in the field weighs 86 pounds per cubic foot. The method of conversion is to divide the percentage of moisture by 6.0465. The figure for density was adopted after making a number of tests throughout the 6-foot profile, using three different methods of sampling. These tests indicated that the soil of the lower sections is more compact than that near the surface, but it is believed that 86 pounds per cubic foot is not far from the average weight of the soil of these plots.

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[2] In this paper this station is called the Prosser experiment farm. Certain features of the investigational work at this station are conducted cooperatively by the Washington State College and the Office of Western Irrigation Agriculture of the Bureau of Plant Industry, U. S. Department of Agriculture. C. C. Wright is detailed from the Office of Western Irrigation Agriculture to conduct these cooperative investigations, of which the subject of the present paper constitutes a part.

[3] BRIGGS, L. J., and McLANE, J. W. THE MOISTURE EQUIVALENTS OF SOILS. U. S. Dept. Agr., Bur. Soils Bul. 45, 23 p., illus. 1907.

TABLE 1.—*Moisture content of the soil profile through the root zone in field E-5, Prosser experiment farm*

Depth of sampling	Moisture equivalent		Wilting point of alfalfa in inches of water per foot of soil	Field-carrying capacity in inches of water per foot of soil	Field-carrying capacity less wilting-point content
	Percentage of dry weight of soil	Inches of water per foot of soil			
Feet					*Inches*
1	16.2	2.68	0.051	3.06	2.55
2	16.2	2.68	.81	3.55	2.74
3	14.2	2.35	.77	3.32	2.55
4	13.3	2.20	.74	2.79	2.05
5	14.3	2.36	1.01	2.78	1.77
6	17.0	2.81	1.02	2.92	1.90

In any attempt to estimate the quantity of water that may be held in the soil of the root zone available for the use of crop plants it is necessary to establish the point of moisture condition that represents the minimum below which the plants are not able to absorb the water they need for normal growth. This point is commonly referred to as the wilting point. Briggs and Shantz [4] have expressed these relationships as follows:

$$\frac{\text{Moisture equivalent}}{1.84 \pm 0.013} = \text{wilting coefficient.}$$

In the investigations reported here the wilting point of the soil was determined by the direct method rather than by computing it from the moisture-equivalent determinations. The method used was to take samples of soil in 1-foot sections to the depth of 6 feet in several alfalfa fields (two locations in field E-5) on the Prosser experiment farm at a time when the alfalfa plants were so severely wilted that they did not recover their normal turgidity at night. These moisture determinations were made in duplicate for each field. The results, expressed as inches of water per foot of soil, are shown in Table 2. When these results are compared with figures for moisture equivalent, given in Table 1, it can be seen that the difference between the moisture equivalent and the wilting point is greater than would be obtained by computation, using the factor proposed by Briggs and Shantz. The figures in the last two columns of Table 2 are believed to represent conditions in soils that are very similar to the samples on which the moisture-equivalent determinations were made as reported in Table 1; consequently the figures in these two columns have been averaged to obtain wilting-point figures for soil of that type. These averages are given in column 4 of Table 1.

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[4] BRIGGS, L. J., and SHANTZ, H. L. THE WILTING COEFFICIENT FOR DIFFERENT PLANTS AND ITS INDIRECT DETERMINATION. U. S. Dept. Agr., Bur. Plant Indus. Bul. 230, 83 p., illus. 1912.

TABLE 2.—*Water content of soil on the Prosser experiment farm at the wilting point for alfalfa*

Depth of sampling	Inches of water per foot of soil in—				
	Field A-4	Field B-4	Field E-4	Field E-5 (1)	Field E-5 (2)
Feet					
1	0.69	0.62	0.64	0.50	0.52
2	.78	.78	.78	.86	.76
3	.78	.78	.72	.68	.87
4	.78	.73	.80	.61	.88
5	.78	.73	.78	.77	1.25
6	.78	.62	1.81	.68	1.36
Average [a]	0.76	0.71	0.92	0.68	0.94

[a] Average per foot of first 4 feet = 0.72; average per foot of all 6 feet = 0.80.

Another essential point to establish in the water relations of the soil is the field-carrying capacity. This may be defined as the quantity of water the soil will hold against gravity. It is significant as indicating the intimate relations that exist between the soil and its suspended solution that the quantity of water so held against the normal pull of gravity is not very much greater than the quantity held against a force one thousand times that of gravity as represented by the moisture equivalent. For the soil represented by the samples reported in Table 1 the field-carrying capacity was determined by taking samples in 1-foot sections to the depth of 6 feet from plots that had recently been heavily irrigated. The field-carrying capacities of Table 1 are based on duplicate samples, separately determined, from each of three plots that had been irrigated about 24 hours before the samples were taken. The quantities of irrigation water applied were, respectively, 11.5, 12, and 18 inches. At the time the irrigation water was put on, the soil was already in a fairly moist condition, so that as a result of irrigation the percolating water passed below the sixth foot.

An estimate of the quantity of water that may be held available for crop plants in the root zone of this soil to the depth of 6 feet may be made by taking the difference between the quantity of water found shortly after irrigation, as shown in column 5 of Table 1 and the quantity found at the time when alfalfa was permanently wilted, as shown in column 4 of that table. These differences, which for each foot are shown in column 6 of the table, and which may be regarded as the capacity of this soil for holding available water, average nearly 2.5 inches per foot for the upper 4 feet and total about 13.5 inches for the 6-foot section. It seems probable that the quantity of water found in the fifth and sixth feet under the wilted alfalfa is rather larger than would be found at the true wilting point for that type of soil. This may have been due to the presence of some fine silt in those sections, or more probably to the fact that the roots of the alfalfa plants were not so abundantly distributed at that depth. On the other hand, the smaller quantity of water found in the first foot was probably due partly to the effect of surface-evaporation losses rather than wholly to the absorption of the alfalfa roots.

A survey of all the results available from soil-moisture determinations made at the Prosser experiment farm leads to the conclusion that in this soil crop plants are not able to withdraw the water to a point much below 0.75 inch per foot, and that probably for the best cropping results irrigation water should be applied when the water content in the zone of active root absorption is not lower than 1 inch per foot. Furthermore, it is apparent that when the soil is dry enough to require irrigation it is possible to store about 2 inches of available water per foot of soil in that root zone.

THE FIELD PLOTS AND THE MEASUREMENT OF THE WATER

The plots used for the studies of water relations were located in field E–5 of the Prosser experiment farm. Each plot was 30 feet square and was surrounded by a dike about 12 inches high. The surface of each plot was carefully leveled to promote the uniform penetration of the irrigation water. These plots or basins, of which there were six, were arranged in a single series. The irrigation water was applied through a wooden flume laid along one side of the series of basins. During the first year of the experiment, 1924, the water was measured over a Cipoletti weir. In 1925 and 1926 a submerged orifice irrigation meter was used. The measuring device was installed in a box set in a branch from a head ditch near the upper end of the series of basins. The volume of water flowing through the weir or meter was regulated to a small but constant stream by means of a turnout gate in the head ditch. Only one basin was watered at a time, and after one basin was irrigated the water was wasted down the flume while the discharge reading was being checked and the figures recorded. It is believed that the arrangement was such that a high degree of accuracy was obtained in the water measurements.

The soil samples for moisture determination were made by taking two cores from each plot with a soil tube. The cores for each foot section were collected in separate soil cans and dried separately, so that each figure for soil moisture is the mean of two determinations. The method of converting the percentages of moisture into inches of water has already been explained.

The data accumulated during the three irrigation seasons 1924 to 1926 afford an opportunity to test the reliability of the methods of measurement and of conversion used in this series of experiments. Fifty-four sets of measurements were made, each involving the quantity of water applied to the soil and the increase in moisture content resulting therefrom. Of these there were 18 cases in which the quantity of water added, together with the quantity already present in the 6-foot section, gave a total of more than 16 inches of water for that section, and consequently it is assumed that some loss occurred through percolation. These cases include the four heaviest applications of the second irrigation in 1924 and the seven 5-inch irrigations of 1925 and 1926.

There remain, then, 36 cases where there is reason to believe that there was little if any percolation loss from the root zone immediately following irrigation, even though the quantity of water in the 6-foot section was in a few instances above 16 inches. For each of these cases there are available the figures for (1) the quantity of water present before irrigation, (2) the quantity of water applied, and (3) the quantity of water present after irrigation. If there were no error either in the measurement of the water applied or in the determination of the water in the soil before and after irrigation, there would be no difference between the two results except for the loss by evaporation during the 24 hours between irrigation and the second sampling of the soil. As a matter of fact, in 24 of the 36 cases the increase in water content reported from the soil samples is less than the estimate as to the quantity of water applied. In 12 cases the quantity reported from the soil samples was larger. The average difference for all cases is −0.16 inch; that is to say, the average increase in soil moisture in the root zone to the depth of 6 feet was 0.16 inch less than the quantity of water applied as irrigation.

RESULTS OF THE INVESTIGATIONS

THE FIRST IRRIGATION OF 1924

The land on which the six basins were located had not previously been irrigated, although water had been used on near-by fields. The basins were prepared in the early summer of 1924, and on July 7 of that year samples were taken from each plot to the depth of 4 feet for moisture determinations. The results of these tests, two sets of samples from each plot, are given in Table 3. It will be seen from this table that the moisture content of the first foot was well below what is regarded as the wilting point for that soil; that of the second foot was approximately the same as the wilting point; and those of the third and fourth feet were above that point. Conditions were very similar in all six plots. The season had been a dry one even for that climate. The precipitation for the three months, April, May, and June, had been only 0.14 inch, while the evaporation from a free-water surface for the same period had been 16.93 inches.

TABLE 3.—*Moisture content of the soil of six plots in field E-5, Prosser experiment farm, July 7, 1924, one day before irrigation*

Plot	Inches of water per foot of soil at depth of—				
	1 foot	2 feet	3 feet	4 feet	Total moisture to 4-foot depth
A	0.75	0.77	0.89	0.99	3.40
B	.55	.90	.93	1.06	3.44
C	.57	.95	.97	1.53	4.02
D	.39	.69	1.01	1.34	3.43
E	.21	.55	.90	1.03	2.69
F	.29	.85	1.04	1.13	3.31
Average	.46	.78	.96	1.18	3.38

On July 8 all six plots were irrigated. The quantity of water applied was estimated as equivalent to 4.5 inches in depth for the areas within the borders. On the following day soil samples were taken in duplicate from each plot for moisture determinations. The results of these are given in Table 4. In comparing the two tables that show the moisture conditions in these plots the day before and the day after irrigation it may be noted that the average increase in moisture content was 4.12 inches from an irrigation of 4.5 inches. In view of the magnitude of the errors of sampling and of water measurement these results are fairly close. Furthermore, there was probably some loss from evaporation in the 24 hours following irrigation and some additional loss from the absorption of water by the dikes surrounding the small plots. With respect to the individual plots, the increase in moisture content ranged from 3.56 to 4.43 inches. The increase in the first foot of soil averaged 2.43 inches and that in the second foot 1.45 inches, leaving only 0.24 inch to pass to the lower levels. In this dry soil, then, the absorptive capacity was approximately 2 inches for each foot. This figure is lower than is given in the last column of Table 1 as the difference between the field-carrying capacity and the moisture content at the wilting point for this type of soil. The differences may be due in part to very local differences in the soil texture and in part to differences in structure, such as might develop after repeated wetting without intervening stirring.

TABLE 4.—*Moisture content of the soil of six plots in field E-5, Prosser experiment farm, July 9, 1924, one day after an irrigation of 4.5 inches*

Plot	Inches of water per foot of soil at depth of—				
	1 foot	2 feet	3 feet	4 feet	Total moisture to 4-foot depth
A	3.05	2.14	1.25	1.39	7.83
B	2.82	2.16	.95	1.07	7.00
C	3.06	2.43	1.29	1.38	8.16
D	2.79	2.52	1.05	.99	7.35
E	2.67	2.02	1.13	1.15	6.97
F	2.95	2.12	1.32	1.31	7.70
Average	2.89	2.23	1.16	1.21	7.50

TABLE 5.—*Moisture content of the soil of six plots in field E-5, Prosser experiment farm, July 29, 1924, three weeks after an irrigation of 4.5 inches*

Plot	Inches of water per foot of soil at depth of—				
	1 foot	2 feet	3 feet	4 feet	Total moisture to 4-foot depth
A	1.62	1.77	1.34	1.36	6.09
B	1.40	1.83	1.46	1.31	6.00
C	1.37	1.71	1.38	1.20	5.66
D	1.55	1.81	1.53	1.34	6.23
E	1.50	1.75	1.42	1.36	6.03
F	1.46	1.70	1.45	1.39	6.00
Average	1.48	1.76	1.43	1.33	6.09

It was desired to observe the changes in the distribution of moisture in the soil and the losses that might occur by evaporation. The surface soil was not disturbed for about three weeks following the sampling on July 9, or until July 29, when another set of samples was taken. The results of the moisture determinations on these samples are given in Table 5. This table shows that during three weeks there was an average net loss of 1.5 inches of water from the 4-foot layer of soil. During the whole month of July for that year the loss by evaporation from a free-water surface was reported as 7.28 inches and the precipitation was 0.19 inch. It should be kept in mind that the quantity here reported as lost from the soil was chiefly evaporation loss. There was practically no plant growth on these plots, and the slight change that occurred in the moisture content of the fourth foot indicates that there was probably very little increase in moisture below that depth. The decrease in moisture for the first foot was 1.41 inches. This is probably as close an approximation of the total evaporation loss as could be expected. The loss from the second foot was 0.47 inch, while the increase of moisture in the third and fourth sections was 0.39 inch. The inference is that approximately 1.5 inches was lost by evaporation, chiefly from the first foot, and that there was some readjustment of moisture from the second foot to the lower layers.

After the samples of soil were taken on July 29 the plots were left undisturbed for another three weeks, or until August 19 and 20, when another set of samples was taken. The moisture contents of these samples are given in Table 6. The average loss for the second period of three weeks was only 0.38 inch as compared with 1.51 inches for the previous three weeks. This loss was fairly well distributed through the 4-foot section. The evaporation loss from a free-water surface for the whole month of August was 6.18 inches. It seems probable that not all of the 0.38 inch reported as lost from the soil escaped by evaporation. The average net loss from the surface foot was 0.09 inch, and, while there may have been some replacement from below, it seems probable that there occurred also some further distribution of moisture downward to the soil below the fourth foot. One of the outstanding features shown by this series of tables is that the uncropped and undisturbed soil gives up its water very slowly, even during the hot, dry summer months.

TABLE 6.—*Moisture content of the soil of six plots in field E-5, Prosser experiment farm, August 19 and 20, 1924, six weeks after an irrigation of 4.5 inches*

Plot	Inches of water per foot of soil at depth of –				
	1 foot	2 feet	3 feet	4 feet	Total moisture to 4-foot depth
A	1. 50	1. 42	1. 20	1. 30	5. 42
B	1. 36	1. 77	1. 40	1. 30	5. 83
C	1. 55	1. 37	1. 17	1. 00	5. 09
D	1. 30	1. 80	1. 42	1. 30	5. 82
E	1. 25	1. 70	1. 40	1. 36	5. 71
F	1. 40	1. 70	1. 40	1. 38	5. 88
Average	1. 39	1. 63	1. 33	1. 27	5. 62

The reports from the Prosser station indicate that during the month of August, 1924, the total precipitation was 0.65 inch, which occurred in two showers. This precipitation no doubt tended to reduce the net loss of water from the soil, but the summer showers of that region are usually so light and so quickly dissipated that their effect on soil-moisture conditions is almost negligible.

The results of the irrigation of July 8 may be briefly summarized as follows: Before irrigation the soil to the depth of 4 feet contained 3.38 inches of water. On the day following an irrigation of 4.5 inches there was a net increase of 4.12 inches, held chiefly in the upper 2 feet of soil. After three weeks there had been a net loss of 1.5 inches, mostly from the first foot, with some movement of water downward from the second foot. During the next three weeks the net loss of water was only 0.38 inch, apparently contributed from the whole 4 feet of soil.

THE SECOND IRRIGATION OF 1924

After observing the changes in moisture conditions that took place during six weeks after an irrigation of 4.5 inches it was decided to extend the depth of sampling and to use larger quantities of water for irrigation. It seemed important to determine by repeated observations how much water would be held by the soil of a deep root zone, in order to estimate how much would be required to leach the root zone if such treatment were necessary as a means of replacing a superconcentrated soil solution. Where the system of irrigation is such that all of the water applied to the soil is held in the root zone to be subsequently dissipated by evaporation or absorbed by plants, it follows that the dissolved salts contained in the irrigation water remain in solution in the root zone. Thus, unless the root zone is leached occasionally, the solution finally becomes so concentrated with respect to these soluble salts that the crop plants are unable to absorb from it the water they require for the processes of growth.

The moisture conditions in the soil of the six basins to the depth of 6 feet were determined by means of duplicate samples taken on August 19 and 20, 1924. The results of these determinations are given in Table 7. This table shows that these plots contained on an average 8.28 inches of water in the soil to the depth of 6 feet. This was distributed fairly uniformly with slightly more in the second foot than in the other sections. From what is known of the water relations of this soil it is evident that it then contained approximately 4 inches of water that would be available to plants having a root system distributed throughout the 6 feet. In other words, it contained more water and had a correspondingly smaller capacity for storing the water of an irrigation than it would have had if covered by a growing crop in need of irrigation. This point should be kept in mind in considering the quantity of water required to leach the root zone of a soil from which nearly all of the available water has been absorbed.

TABLE 7.—*Moisture content of the soil of six plots in field E 5, Prosser experiment farm, August 19 and 20, 1924, before the second irrigation*

Plot	Inches of water per foot of soil at depth of—						
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	Total moisture to 6-foot depth
A	1.50	1.42	1.20	1.30	1.25	1.22	7.89
B	1.36	1.77	1.40	1.30	1.16	1.31	8.30
C	1.55	1.37	1.17	1.00	1.19	1.34	7.62
D	1.30	1.80	1.42	1.30	1.55	1.48	8.85
E	1.25	1.70	1.40	1.36	1.30	1.28	8.29
F	1.40	1.70	1.40	1.38	1.50	1.36	8.74
Average	1.39	1.63	1.33	1.27	1.32	1.33	8.28

On August 21 four of the plots were irrigated, as follows: To plot A, 5 inches of water was applied; to plot B, 6.5 inches; to plot C, 11.5

inches; and to plot D, 8.8 inches. On August 22 the two remaining plots were irrigated, plot E being given 18 inches and plot F 12 inches. The soil absorbed the water readily, but the surfaces of plots E and F were so wet on the day following irrigation that the soil samples could not be taken until the second day. The first four plots were sampled in duplicate to the depth of 6 feet on August 22, and the last two were sampled in the same way on August 24. The results of the moisture determinations on these samples are given in Table 8.

TABLE 8.—*Moisture content of the soil of six plots on field E-5, Prosser experiment farm, August 22 and 24, 1924, immediately after the application of different quantities of irrigation water*

Plot	Inches of water per foot of soil at depth of—						
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	Total moisture to 6-foot depth
A	2.85	3.28	2.07	1.56	1.85	1.40	13.01
B	3.27	3.46	2.81	1.85	1.58	1.51	14.48
C	3.39	3.72	3.05	2.61	2.63	2.12	17.52
D	2.80	3.65	3.24	2.86	2.28	2.00	16.83
E	3.30	3.24	3.31	3.14	3.15	3.10	19.24
F	2.48	3.68	3.60	2.64	2.56	3.54	18.50
Average	3.01	3.51	3.01	2.44	2.34	2.28	16.60

In view of the fact that each plot was given a different quantity of water it is necessary to confine the comparison between conditions before and after irrigation to the individual plots, though the averages for each foot for all six plots are given in both tables.

For plot A, to which 5 inches of water was applied, the total increase in water was 5.12 inches, of which 4.08 inches was held in the first 3 feet. It is not clear that there was immediately any effective penetration below the sixth foot. For plot B, to which 6.5 inches was applied, the total increase reported was 6.18 inches, of which 5.01 inches was held in the first 3 feet. Here, again, there appears not to have been any effective percolation beyond the sixth foot at least since the moisture content of that layer of soil was still well below its field-carrying capacity, and the results indicate that it was increased by only 0.2 inch at the end of 24 hours after irrigation.

The application of 11.5 inches of water to plot C increased the total moisture supply by 9.9 inches. This would indicate that 1.6 inches had passed below the sixth foot in 24 hours, and it might be assumed that some leaching action had resulted.

From what is known of the process of leaching, based on laboratory work with soil columns as well as on field samplings, it is believed that the application of water to the surface of the soil is followed by a displacement downward of the original soil solution rather than the diffusion of the added water through the solution. These observations lead to the view that the water that percolates, for example, from the fourth foot into the fifth foot is largely composed of the soil solution originally held in the upper layers of the soil. Consequently, where there is evidence of a movement of water, as from the sixth foot downward, it is assumed that such water contains not only the salt originally held in solution in the moisture of the sixth foot but also some additional salt brought down in solution from the upper soil.

On plot D the application of water was 8.8 inches, and the resulting increase in water content of the 6 feet of soil was 7.98 inches. Of this amount, 6.73 inches was retained, at least temporarily, in the first 4 feet of soil; and while the moisture content of the fifth and sixth feet was increased appreciably, it is not clear that there was much effective percolation below the sixth foot.

The irrigation of plot E was very heavy, 18 inches of water being used. This made the ground so soft that samples could not be taken until the second day after irrigation. The test of these samples indicated that the soil had been well filled with water, there being more than 3 inches in each foot, with a total increase of 10.95 inches for the 6-foot section. The difference between that quantity and the 18 inches applied was nearly as large as the quantity of water held in the section before irrigation. It seems probable that the solution originally present was very largely replaced by the water added, and consequently that a single irrigation of 18 inches on this soil would result in complete and effective leaching of the root zone to 6 feet at least.

Finally plot F was irrigated with 12 inches of water, of which 9.76 inches was retained in the upper 6 feet. It may be assumed that the original solution was almost completely displaced from the first 4 feet of soil and that approximately 3 inches of water, containing a substantial proportion of the dissolved salts originally in the root zone, passed below the limit of observation.

It may be recalled from Table 1 that the soil of these basins showed a moisture-equivalent capacity of 15.08 inches for the first 6 feet; that is to say, the soil of that section when tested in the laboratory held that equivalent of water against a force one thousand times that of gravity. The field observations as reported in Table 8 indicate that the soil may hold, for some hours at least, as much as 18 inches of water against the force of gravity in the 6-foot section.

Following the sampling of August 22 and 24 the plots were left undisturbed for a week, or until August 30. On that date another set of duplicate samples was taken in order to determine what loss or redistribution of moisture had taken place. The results for this set of samples are given in Table 9. For the series of plots as a whole the average moisture content for the 6-foot section had decreased by 2.52 inches. This decrease was distributed as follows: From the first 2 feet, 0.73 inch for each; from the third foot, 0.57 inch; from the fourth foot, 0.27 inch; from the fifth foot, 0.19 inch; while from the sixth foot only 0.03 inch was lost.

The losses reported for the individual plots are less consistent than the averages for the 1-foot sections. They ranged from 1.35 inches for plot A to 4.23 for plot F. It is not apparent why the losses indicated for plot B, 2.91 inches, and for plot F, 4.23 inches, should be so much above the average. These are probably to be explained as examples of the aberrant results caused by very local differences in the texture of the soil associated with the isolated strata of fine silt referred to previously. A critical examination of Table 9 shows that if the lower 3 feet of plots A and B are eliminated from consideration, the average water content of the remaining plot sections is equivalent to 2.49 inches per foot, while the average moisture equivalent as taken from Table 1 is 2.51 inches per foot. The reason for eliminating the lower sections of plots A and B from this comparison is that the quantity of irrigation water applied to these two plots was evidently not sufficient to saturate the whole 6-foot section. The comparison just made appears to warrant the assumption that the moisture equivalent of this soil as determined in the laboratory affords a fair basis for estimating its field-carrying capacity after conditions of equilibrium have become established. This is substantially the conclusion arrived at by Burr and Russel as a result of an extensive series of observations on the water relations of Nebraska soils.[5]

TABLE 9.—*Moisture content of the soil of six plots on field E-5, Prosser experiment farm, August 30, 1924, one week after the application of different quantities of irrigation water*

Plot	Inches of water per foot of soil at depth of—						
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	Total moisture to 6-foot depth
A	2.29	2.21	2.08	1.93	1.89	1.26	11.66
B	2.18	2.73	1.99	1.53	1.66	1.48	11.57
C	2.21	3.47	2.59	2.46	2.02	2.63	15.38
D	2.30	2.63	2.44	2.22	2.79	2.40	14.78
E	2.47	3.25	3.20	2.26	2.44	3.18	16.80
F	2.25	2.42	2.34	2.61	2.10	2.55	14.27
Average	2.28	2.78	2.44	2.17	2.15	2.25	14.08

Finally, after the sampling of August 30 the plots were left undisturbed until October 1, a period of six weeks from the last irrigation. During the month of September the precipitation amounted to 0.69 inch, occurring in three showers, and the evaporation from a free-water surface was 3.71 inches for the month. The moisture condition in the plots on October 1, 1924, is shown in Table 10. During the five weeks following August 30 the average loss of water from the 6-foot section of each plot was 2.32 inches. This loss as shown by the averages was fairly well distributed throughout the upper 5 feet. The water content of the sixth foot was practically unchanged. It seems altogether probable that the larger part of the water lost was dissipated by evaporation, although there was doubtless some movement downward also. There was no crop growth to absorb moisture from the soil, though a few small weeds that were permitted to survive may have used some water.

It becomes apparent on comparing in detail the results presented in Tables 9 and 10 that the dissipation of water occurred by way of vaporization well below the surface of the soil; in other words, evaporation was not confined to the surface layer of soil. It appears to have been assumed by some investigators that the loss of water from the soil takes place as a result of evaporation at the soil surface, accompanied by the rise of water by capillarity from the lower soil to replace the evaporation losses. It is not to be questioned that in some situations the evaporation of water is localized at or near the surface of the soil and that the supply to be vaporized in that area is maintained by capillary movement from the saturated subsoil. In such situations there is often formed a crust of salt on the soil surface, or a high proportion of soluble salt is found in a thin layer of surface soil. Such conditions are observed usually only where the subsoil is saturated with water not far below the surface and where the condition of saturation is maintained by hydrostatic pressure. When there is no zone of saturated subsoil within a very few feet of the surface the vaporization of soil moisture appears to take place well down in the soil, as well as at or near the surface. Under such conditions there is an appreciable circulation of air throughout the root zone, and in a moist soil the soil atmosphere is approximately saturated with water vapor. There is some basis both in laboratory experiments and in field observations for the view that the distribution of water through the root zone in the direction of establishing conditions of equilibrium takes place quite as much by alternate vaporization and condensation as by the capillary movement of liquid water.

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[5] BURR, W. W. and RUSSEL, J. C. REPORT OF CERTAIN INVESTIGATIONS ON THE CENTRAL NEBRASKA SUPPLEMENTAL IRRIGATION PROJECT. Nebr. Dept. Public Works Bien. Rpt. (1923/24) 15: 199-240, illus. [1925].

TABLE 10.—*Moisture content of the soil of six plots on field E-5, Prosser experiment farm, October 1, 1924, six weeks after the application of different quantities of irrigation water*

Plot	Inches of water per foot of soil at depth of						
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	Total moisture to 6-foot depth
A	1.93	1.93	1.58	1.69	1.53	1.73	10.39
B	1.93	1.93	1.53	1.50	2.08	2.11	11.08
C	2.26	2.49	2.10	1.81	1.80	2.57	13.03
D	1.89	2.76	1.97	1.73	1.55	2.37	12.27
E	1.73	2.37	2.02	1.58	1.73	2.84	12.27
F	2.00	2.36	1.75	1.62	1.76	2.03	11.52
Average	1.96	2.31	1.82	1.65	1.74	2.27	11.76

The outstanding result of the observations reported in Table 10 is that six weeks after irrigation—a period of dry, warm weather—the soil of these plots still held nearly 12 inches of water in the first 6 feet, and that probably nearly 8 inches of that water could be regarded as available for the use of plants.

THE IRRIGATIONS OF 1925

It was desired, for the season of 1925, to determine what changes in moisture conditions in the six plots in field E-5 would follow from a regular sequence of irrigations on uncropped land. For this purpose the plots were grouped into adjacent pairs and to each pair was given a different quantity of water, though the number of irrigations was the same for all. The plots were irrigated seven times from April 27 to September 2, inclusive, the interval between irrigations being about three weeks. Plots A and B were given 4 inches of water at the first irrigation and 2 inches at each subsequent irrigation. Plots C and D were given 3.5 inches and plots E and F were given 5 inches at each irrigation throughout the season. The plots were not cropped, and the weeds were kept down by pulling or hoeing. Samples of soil were taken in duplicate from each plot the day before and again the day after each irrigation.

During the period from May 1 to August 31, inclusive, the precipitation amounted to 0.94 inch. This occurred in the form of light showers, and it is believed that these did not affect materially the moisture conditions of the soil in the plots under observation. During the same period the total evaporation from a free-water surface was 24.76 inches.

The moisture conditions for the season of 1925, the averages for plots A and B to the depth of 6 feet are given in Table 11. The date given in the first column of the table is the date on which the plots were irrigated. The water content opposite the word "before" is that found the day before irrigation, while that opposite the word "after" is that found the day after irrigation. The data for the irrigation of April 27 are not included in the averages because a larger quantity of water was used then than later.

TABLE 11.—*Moisture content of the soil in plots A and B, field E–5, Prosser experiment farm, for the season of 1925, before and after each of seven irrigations, with a total application of 16 inches of water*

Date	Inches of water per foot of soil at depth of—						Total moisture to 6-foot depth	Increase	Decrease
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet			
Apr. 27:									
Before	1.76	1.89	1.55	1.50	1.81	1.96	10.47		
After	2.97	3.52	1.76	1.81	2.05	1.63	13.74	3.27	
May 20:									
Before	2.14	2.39	2.04	1.70	1.89	1.67	11.83		1.91
After	2.70	2.64	2.26	1.95	2.13	1.97	13.65	1.82	
June 12:									
Before	2.14	2.25	1.97	1.70	1.96	2.32	12.34		1.31
After	2.70	2.50	2.19	1.90	2.02	2.20	13.51	1.17	
July 2:									
Before	2.09	1.99	1.87	1.93	2.24	2.36	12.48		1.03
After	2.97	2.27	1.87	1.89	2.26	1.83	13.09	.61	
July 21:									
Before	1.85	2.05	1.80	1.71	2.03	1.74	11.18		1.91
After	2.88	2.65	1.71	1.60	2.06	1.80	12.70	1.52	
Aug. 12:									
Before	1.72	2.05	1.73	1.80	2.18	1.74	11.22		1.48
After	3.03	2.87	2.03	1.83	1.99	1.97	13.72	2.50	
Sept. 2:									
Before	1.92	2.33	1.89	1.65	2.02	1.96	11.77		1.95
After	2.89	2.54	2.08	1.79	2.08	1.90	13.28	1.51	
Average:[a]									
Before	1.98	2.18	1.88	1.75	2.05	1.96	11.80		
After	2.86	2.58	2.02	1.83	2.09	1.94	13.32		
Increase or decrease[b]	+.88	+.40	+.14	+.08	+.04	−.02	+1.52		

[a] The 4-inch irrigation of Apr. 27 is omitted from the averages.

[b] The difference between the average before irrigation and the average immediately afterwards.

While the detailed figures in this and the following tables show some irregularities characteristic of field observations of moisture conditions, it is believed that the averages at the bottom of the table give a fairly true picture of the changes that occurred. It will be noted that whereas the average application of water was estimated as 2 inches in depth for each plot, the average increase in moisture content resulting from irrigation is given as 1.52 inches. It seems hardly probable that the difference of 0.48 inch was lost by evaporation between the time of irrigation and the time of sampling the following day. The inference is that a part of the discrepancy may have been due to unavoidable errors either in measuring the irrigation water or in computing the moisture content of the soil. It does not seem probable that for these two plots there was an appreciable loss of water by percolation below the sixth foot, though there may have been during the season a slight increase in moisture below the sixth foot as a result of capillary movement or vaporization.

The averages of the increases in moisture content resulting from irrigation show a consistent decline from the first to the fifth foot. It is doubtful, however, whether the changes shown for the second 3 feet are to be regarded as of sufficient magnitude to be significant. In other words, this evidence appears to indicate that with irrigations of only 2 inches, even without crop absorptions, there was not much effect below the third foot.

The moisture conditions for the season for plots C and D are shown in Table 12. These plots were irrigated seven times with 3.5 inches of water each time. The average increase in moisture content for the 6-foot section is given as 3.1 inches. Here again the average increase shown is less than the average estimated application of water. The average increase in moisture content for each successive section shows a progressive decline, with a very slight increase for the fifth foot and an insignificant difference for the sixth foot. The indications are that there was no leaching action, although at the end of the season the moisture content of the whole section was slightly higher than at the beginning.

TABLE 12.—*Moisture content of the soil in plots C and D, field E 5, Prosser experiment farm, for the season of 1925, before and after each of seven irrigations, with a total application of 24.5 inches of water*

Date	Inches of water per foot of soil at depth of—						Total moisture to 6-foot depth	Increase	Decrease
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet			
Apr. 27:									
Before	1.78	2.20	1.94	1.71	1.76	1.94	11.33		
After	3.06	3.66	2.47	2.19	1.65	1.77	14.80	3.47	
May 20:									
Before	1.80	2.46	1.86	1.77	1.82	1.87	11.58		3.22
After	2.63	3.50	2.74	1.90	1.74	1.90	14.41	2.83	
June 12:									
Before	2.03	2.83	2.29	2.17	1.81	2.17	13.30		1.11
After	2.60	3.15	2.83	2.56	2.41	2.46	16.01	2.71	
July 2:									
Before	2.20	2.68	2.41	2.24	1.99	2.42	13.94		2.07
After	3.31	3.41	3.28	2.39	2.13	1.90	16.42	2.48	
July 21:									
Before	1.83	2.34	2.29	1.69	1.87	2.10	12.12		4.30
After	3.16	3.51	2.78	2.06	1.73	2.04	15.28	3.16	
Aug. 12:									
Before	2.07	2.42	2.10	1.73	1.65	2.20	12.17		3.11
After	2.97	3.32	2.88	2.42	2.32	2.38	16.29	4.12	
Sept. 2:									
Before	1.92	2.61	2.29	1.93	1.94	2.33	13.02		3.27
After	3.28	3.49	2.74	2.15	2.01	2.26	15.93	2.91	
Average:									
Before	1.95	2.51	2.17	1.89	1.83	2.15	12.49		
After	3.00	3.43	2.82	2.24	2.00	2.10	15.59		
Increase or decrease [a]	+1.05	+.92	+.65	+.35	+.17	−.05	+3.10		

[a] The difference between the average before irrigation and the average immediately afterwards.

The conditions in plots E and F are shown in Table 13. These plots received 5 inches of water at each of seven irrigations. The average increase in moisture content reported for each irrigation was 3.62 inches. It seems probable that there was some percolation or downward movement of water below the sixth foot. There was also an appreciable increase in moisture content in the 5-foot and 6-foot layers after each irrigation. These 5-inch irrigations, with no crop growth to use the water, appear to have been adequate to produce some leaching effect.

TABLE 13.—*Moisture content of the soil in plots E and F, field E–5, Prosser experiment farm, for the season of 1925, before and after each of seven irrigations, with a total application of 35 inches of water*

Date	Inches of water per foot of soil at depth of—						Total moisture to 6-foot depth	Increase	Decrease
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet			
Apr. 27:									
Before	1. 51	2. 13	2. 17	1. 76	1. 69	2. 34	11. 60		
After	2. 40	2. 90	2. 82	1. 96	1. 69	2. 44	14. 21	2. 61	
May 20:									
Before	2. 05	2. 39	2. 05	1. 76	1. 88	1. 84	11. 97		2. 24
After	2. 70	3. 42	3. 69	2. 68	2. 40	2. 86	17. 75	5. 78	
June 12:									
Before	2. 45	2. 75	2. 63	2. 41	2. 40	2. 58	15. 22		2. 53
After	2. 22	3. 60	3. 35	2. 58	2. 50	3. 02	17. 27	2. 05	
July 2:									
Before	1. 97	2. 43	2. 91	2. 14	2. 31	2. 65	14. 41		2. 86
After	2. 99	3. 00	3. 55	2. 90	1. 95	2. 97	17. 36	2. 95	
July 21:									
Before	1. 83	2. 98	2. 64	1. 93	1. 97	2. 51	13. 86		3. 50
After	3. 07	3. 60	3. 00	2. 30	2. 60	2. 70	17. 27	3. 41	
Aug. 12:									
Before	1. 92	2. 39	2. 33	1. 91	1. 88	2. 76	13. 19		4. 08
After	2. 96	4. 14	3. 50	2. 80	2. 12	2. 63	18. 15	4. 96	
Sept. 2:									
Before	1. 71	2. 83	2. 52	2. 52	1. 89	2. 37	13. 84		4. 31
After	3. 03	4. 02	3. 22	2. 57	1. 90	2. 65	17. 39	3. 55	
Average:									
Before	1. 92	2. 56	2. 46	2. 06	2. 00	2. 44	13. 44		
After	2. 77	3. 53	3. 30	2. 54	2. 17	2. 75	17. 06		
Increase [a]	+. 85	+. 97	+. 84	+. 48	+. 17	+. 31	+3. 62		

[a] The difference between the average before irrigation and the average immediately afterwards.

THE IRRIGATIONS OF 1926

In 1926 the irrigation experiments were continued on the same pairs of basins that were used in 1925. The same number of irrigations were given, and the same quantities of water were used except that on plots A and B the first irrigation was of 2 inches, like the later ones, instead of 4 inches as in 1925. Furthermore, in 1926 all the plots were planted to corn, while they were kept fallow in 1925. The corn crop did not do well and the plants did not make many ears. The whole crop was harvested for silage, and the green weights of yield were as follows: For plots A and B, 2,532 pounds per acre; for plots C and D, 3,288 pounds per acre; and for plots E and F, 3,000 pounds per acre. For May to August, inclusive, the precipitation was 2.32 inches, and the evaporation from a free water surface was 24.98 inches.

TABLE 14.—*Moisture content of the soil in plots A and B, field E-5, Prosser experiment farm, for the season of 1926, before and after each of seven irrigations, with a total application of 14 inches of water*

Date	Inches of water per foot of soil at depth of—							Increase	Decrease
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	Total moisture to 6-foot depth		
Apr. 28:									
Before	1.98	2.03	2.10	1.96	2.25	1.80	12.12		
After	3.47	3.65	2.64	2.03	2.10	1.60	15.49	3.37	
May 15:									
Before	2.46	2.35	1.96	2.25	2.55	2.60	14.17		1.32
After	3.07	3.30	2.12	2.00	2.20	2.05	14.74	.57	
June 6:									
Before	2.34	2.45	2.34	1.89	2.15	2.27	13.44		1.30
After	4.07	3.19	2.56	2.10	1.87	2.07	15.86	2.42	
June 26:									
Before	1.81	1.70	2.01	1.85	1.80	2.12	11.29		4.57
After	2.29	2.78	2.04	1.81	1.84	1.94	12.70	1.41	
July 17:									
Before	1.12	1.71	1.41	1.49	1.90	1.94	9.57		3.13
After	2.94	2.35	1.92	1.73	2.68	2.07	13.69	4.12	
Aug. 7:									
Before	1.04	1.57	1.68	1.80	2.21	2.01	10.31		3.38
After	2.82	1.96	1.56	1.61	2.18	1.93	12.06	1.75	
Aug. 30:									
Before	1.50	1.73	1.55	1.59	1.93	1.75	10.05		2.01
After	2.29	2.73	1.90	1.84	1.82	1.82	12.40	2.35	
Average:									
Before	1.75	1.93	1.86	1.83	2.11	2.07	11.56		
After	2.99	2.85	2.11	1.87	2.10	1.93	13.85		
Increase or decrease[a]	+1.24	+.92	+.25	+.04	−.01	−.14	+2.29		

[a] The difference between the average before irrigation and the average immediately afterwards.

The moisture conditions for plots A and B for 1926 are shown in Table 14. It was estimated that these plots received 2 inches of water at each irrigation, yet the average increase in water content as a result of irrigation is shown in the table to have been 2.29 inches. The figures for the average increases for each foot of soil show that there was little change in moisture content below the third foot. The fact that the average increase in moisture was greater than the average quantity applied as irrigation can be explained only as a discrepancy due to errors of water measurement or of soil sampling.

A comparison of conditions on these plots in 1925, as shown in Table 11, with those of 1926, as shown in Table 14, shows something of the effect of the corn crop on the moisture supply. Notwithstanding a higher average increase of moisture for each irrigation, the soil contained less water at the end of the crop season. This decrease in moisture supply was most pronounced in the upper 4 feet. There was very little difference in the lower layers. The data available do not warrant an attempt to estimate the quantity of water used by the crop in comparison with the quantity lost by direct evaporation. However, it is apparent from the figures given for the first and the second foot for 1926 that during the latter part of the season the soil was much drier than for the corresponding period of 1925.

TABLE 15.—*Moisture content of the soil in plots C and D, field E–5, Prosser experiment farm, for the season of 1926, before and after each of seven irrigations, with a total of 24.5 inches of water*

Date	Inches of water per foot of soil at depth of—						Total moisture to 6-foot depth	Increase	Decrease
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet			
Apr. 28:									
Before	2.00	1.98	1.86	2.41	1.68	1.71	11.64		
After	3.03	3.65	3.60	1.81	1.71	1.71	15.51	3.87	
May 15:									
Before	2.54	3.08	2.27	1.85	1.79	2.35	13.88		1.63
After	3.38	4.02	2.98	1.60	1.92	2.17	16.07	2.19	
June 6:									
Before	1.84	3.18	2.25	1.96	1.94	2.54	13.71		2.36
After	2.53	3.97	3.23	2.42	2.06	3.06	17.27	3.56	
June 26:									
Before	2.65	2.25	2.19	1.75	1.71	1.97	12.52		4.75
After	2.55	3.60	2.70	2.26	2.11	2.13	15.35	2.83	
July 17:									
Before	1.01	2.13	2.00	1.79	1.61	1.92	10.46		4.89
After	3.36	3.61	2.68	2.08	1.98	2.15	15.86	5.40	
Aug. 7:									
Before	1.24	1.87	1.91	1.54	1.67	1.79	10.02		5.84
After	2.86	2.49	2.24	2.28	1.96	1.96	13.79	3.77	
Aug. 30:									
Before	1.44	2.27	1.70	1.54	1.65	2.16	10.76		3.03
After	2.25	3.72	2.67	2.04	1.83	2.14	14.65	3.89	
Average:									
Before	1.82	2.39	2.03	1.83	1.72	2.06	11.86		
After	2.85	3.58	2.87	2.07	1.94	2.19	15.50		
Increase [a]	+1.03	+1.19	+.84	+.24	+.22	+.13	+3.64		

[a] The difference between the average before irrigation and the average immediately afterwards.

On plots C and D the moisture conditions as shown in Table 15 were again different from those of 1925. The average increase in moisture content was 3.64 inches, while each irrigation was only 3.5 inches. At the close of the season the soil contained less water than at the beginning. There was apparently some change in moisture content, resulting from irrigation, as far down as the fifth foot and possibly even to the sixth foot. The largest changes were confined to the upper 3 feet. It is evident again in this table that the absorption of water by the corn crop tended to lower the moisture content of the upper layers of the soil.

The moisture conditions for plots E and F for 1926 are shown in Table 16. The average increase in moisture content resulting from irrigation was 3.87 inches as compared with an estimated application of 5 inches. It is noticeable that for the whole 6-foot section there was not a decrease in moisture content during the season. In other words, 35 inches of irrigation water was apparently sufficient to meet the evaporation losses and the growth requirements of the light crop of corn. It is quite possible also that there may have been some leaching action, since the moisture content of the sixth foot appears to have been not far below what has been estimated as its field-carrying capacity.

DISCUSSION OF RESULTS

The soil used in the experiments here reported had a moisture equivalent of 16 per cent. A series of careful measurements showed that it could hold in the upper 6 feet about 16 inches of water, of which about 12 inches could be used by plants. It was found that 1 inch of water added to the soil would increase the moisture content of 1 foot by 6 per cent or of the 6-foot section by 1 per cent. This is approximately the same relationship that has been found by the writers to obtain in other types of irrigated soil and that has been reported by other investigators.[6] It may be assumed as a general rule that with a wide range of soil types 1 inch of water in 1 foot of soil is equivalent to 6 per cent of the dry weight of the soil.

TABLE 16.—*Moisture content of the soil in plots E and F, Prosser experiment farm, for the season of 1926, before and after each of seven irrigations, with a total of 35 inches of water*

Date	Inches of water per foot of soil at depth of—							Increase	Decrease
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	Total moisture to 6-foot depth		
Apr. 28:									
Before	2.35	1.98	2.00	1.80	1.60	2.02	11.75		
After	3.15	3.88	3.12	1.84	1.59	2.12	15.70	3.95	
May 15:									
Before	1.88	3.07	2.69	1.88	1.66	2.70	13.88		1.82
After	3.02	4.00	3.14	2.62	1.97	2.00	16.75	2.87	
June 6:									
Before	1.82	2.86	2.38	2.30	2.59	2.89	14.84		1.91
After	2.75	3.30	3.01	2.85	2.15	2.69	16.75	1.91	
June 26:									
Before	1.36	3.00	2.44	1.96	2.36	2.60	13.72		3.03
After	2.81	3.27	3.34	2.76	2.13	2.44	16.75	3.03	
July 17:									
Before	0.89	2.78	2.14	1.81	1.65	1.45	10.72		6.03
After	3.10	3.64	3.09	2.38	2.10	2.43	16.74	6.02	
Aug. 7:									
Before	1.62	2.55	2.02	1.77	1.82	2.81	12.59		4.15
After	2.91	3.18	2.63	2.24	2.02	2.31	15.29	2.70	
Aug. 30:									
Before	1.73	2.28	2.07	2.09	1.94	2.41	12.52		2.77
After	2.44	4.22	3.03	2.87	3.31	3.23	19.10	6.58	
Average:									
Before	1.66	2.65	2.25	1.94	1.95	2.41	12.86		
After	2.88	3.64	3.05	2.51	2.18	2.46	16.73		
Increase [a]	+1.22	+.99	+.80	+.57	+.23	+.05	+3.87		

[a] The difference between the average before irrigation and the average immediately afterwards.

There appears to be also a fairly close relationship between the moisture equivalent of a soil as determined in the laboratory and the field-carrying capacity. That is to say, a soil that has been pulverized and saturated with water as it is prepared for a test in the centrifugal machine will hold against a force one thousand times that of gravity about the same proportion of water as will be held against the force of gravity by the same soil as it occurs in the field.

There is some question as to whether it is safe to assume that the wilting coefficient as computed by dividing the moisture equivalent by 1.84 correctly represents the quantity of water that can be withheld by the soil from absorption by plants. To put the matter in another way: If it is assumed that the moisture equivalent may be taken as approximately the same as the field-carrying capacity, then the wilting coefficient may be computed by dividing the moisture equivalent by 1.84. If the wilting coefficient as determined by that computation be taken as the lower limit of available moisture, then the capacity of the soil for holding available water would be taken as the difference between the moisture equivalent and the wilting coefficient. This would indicate that if the soil were wet up to its field-carrying capacity, only about 45 per cent of the water would be available to crop plants. In these experiments it has been shown that the proportion of available water is not far from 70 per cent of the field-carrying capacity.

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[6] ISRAELSEN, O. W., and WEST, F. L. WATER-HOLDING CAPACITY OF IRRIGATED SOILS. Utah Agr. Expt. Sta. Bul. 183, 24 p., illus. 1922.

These considerations are, however, incidental to the chief purpose of the present inquiry. This purpose was to determine the water-holding capacity of the soil of the root zone with reference to leaching action. It is coming to be generally recognized that in irrigated soil the root zone must be leached from time to time in order to remove the salts brought in by the irrigation water. Irrigation waters differ from rain water in that they contain appreciable quantities of dissolved salts. These salts are not, in a large measure, absorbed by plants, and consequently they remain in the soil solution of the root zone. If the system of irrigation is such that all of the water applied is held in the root zone to be absorbed by plants or dissipated by vaporization, then each successive irrigation augments the salt content of the soil solution in the root zone. In time the soil solution becomes so concentrated with dissolved salts that crop plants are not able to absorb from it the water they need for the processes of growth. The only way that dissolved salts can be removed from the root zone is by leaching. This process involves the application of a quantity of irrigation water sufficient to exceed the field-carrying capacity and thereby displace the concentrated solution of the root zone.

Some irrigation waters contain as much as 1,000 parts per million of salts of high solubility. An acre-foot of water weighs approximately 2.72 million pounds. Consequently an acre-foot of water containing 1,000 parts per million of highly soluble salts carries 2,720 pounds of such salt. It is not uncommon in irrigated regions having a long growing season to use 3 acre-feet of water on each acre of land, applied in 8 to 10 irrigations. This means that the average application is 4 inches or less for each irrigation. This quantity of water is barely sufficient to supply the needs of the crop and the unavoidable evaporation losses. Consequently there is no leaching action and all of the salt brought in by the 3 acre-feet of irrigation water remains in the soil of the root zone, for the most part dissolved in the soil solution. Under such conditions as those just described the annual increase of salt in the root zone would be at the rate of 7,000 pounds (3.5 tons) per acre. It is obvious that such a course of events could not be continued for many years without disastrous results.

In the soil that has been used in the experiments here described the field-carrying capacity to the depth of 6 feet has been found to be about 16 inches of water or 1⅓ acre-feet. When the water available to crop plants has been exhausted the root zone contains less than 6 inches of water. If it is assumed that the soil is irrigated when its supply of water is diminished to about 6 inches in the upper 6 feet, then the soluble salts of the root zone are largely concentrated in this 6 inches of water. If there were 7,000 pounds of salts dissolved in 6 acre-inches of water, the concentration of the solution would be about 0.6 per cent. From this it becomes clear that if it is necessary to use salty water for irrigation it is imperative that it be used in quantities sufficient to leach the root zone from time to time. Furthermore, it is obvious that the more salt there is in irrigation water the more water must be used in order to keep the root zone leached and thus prevent the superconcentration of the soil solution.

SUMMARY

The soil used in these experiments is classed as a sandy loam having a moisture equivalent of about 16 per cent. It has been found that with this soil, as with many other types, the weight relationships are such that 1 inch of water in 1 foot of soil is equivalent to 6 per cent of the dry weight of the soil.

When the soil has been thoroughly irrigated it is found to hold about 16 inches of water in the first 6 feet. When the available supply of water has been absorbed, as by a crop of alfalfa, it still contains about 5 inches of water in the first 6 feet.

It is not good farming practice to postpone irrigation until all of the available water has been absorbed by the crop; consequently, when the indications are that irrigation is needed there probably remains at least 10 inches of water within the root zone. The application of 4 to 6 acre-inches of water at each irrigation during the growing season probably does not result in any effective leaching of the root zone. When the soil is very dry it may hold an irrigation of as much as 10 inches without leaching.

The indications are that when the soil contains less water than its field-carrying capacity, the loss of water by vaporization takes place not only at the soil surface but also well down in the soil. It seems probable also that the movement of water through the soil in the direction of establishing conditions of moisture equilibrium, when the moisture content is below the field-carrying capacity, takes place not so much by capillarity as by vaporization and subsequent condensation.

With this soil, at least, the proportion of water available to crop plants (i. e., to alfalfa) is about 70 per cent of the field-carrying capacity, rather than about 45 per cent, as would be inferred by computing the wilting coefficient from the moisture equivalent.

In order to leach the root zone and thus remove the highly soluble salts brought in by the irrigation water, it is necessary to apply more water than is customarily used to supply the needs of crop plants. If the irrigation water is salty, the root zone must be leached more frequently than when purer water is used.

DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION: A DIETARY STERILITY ASSOCIATED WITH VITAMIN A DEFICIENCY[1]

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INTRODUCTION

In previous communications conclusive evidence was presented of the existence of a specific vitamin for reproduction, designated as vitamin E (*6*, *7*, *8*).[2] [3] Among rats on a diet furnishing an abundance of fat-soluble vitamins A and D in the form of cod-liver oil female sterility was observed, characterized by resorption of the fetus during gestation, which was prevented by the addition of wheat oil, or small amounts of unsaponifiable matter therefrom, to the sterility-producing diet. The resorption of the embryos was determined by the character of the gestation curve during the period of advanced pregnancy and by a post-mortem examination of the uterine horns (*10*).

The composition of the ration which has never failed to produce sterility is as follows: Skim-milk powder, 50 per cent; agar-agar, 2; ferric citrate, 0.2; Harris yeast, 1; cod-liver oil, 2; dextrin, 44.8. The simple expedient of adding 3 per cent wheat oil (replacing an equivalent amount of dextrin in the ration) to such a dietary régime resulted not only in continuous fertility but also in normal lactation, and in healthy, vigorous succeeding generations. Fifth-generation animals that made growth far superior to that indicated by the Donaldson standard have been secured on the diet containing wheat oil, which has been found to be the most potent source of vitamin E (*10*).

In a recent study of vitamin E potency of butter fat (*12*), the writer has reported experimental results on a different dietary régime, showing that continuous fertility can be much more certainly assured by supplementary administrations of cod-liver oil (to furnish vitamins A and D) to pregnant females receiving 5 per cent butter fat in the ration as the only source of all known fat-soluble vitamins. The need of vitamin A as well as vitamin E in fertility was anticipated but no conclusive evidence was then available.

EXPERIMENTAL DATA

In the present report positive evidence is submitted associating female sterility with vitamin A deficiency, which is, so far as the writer has been able to determine, identical in every respect with that produced by a deficiency of vitamin E. The ration used in the experimental feeding (No. 835) was composed of the following: Skim-

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[1] Received for publication May 14, 1928; issued October, 1928. Research Paper No. 63, Journal Series, University of Arkansas. This report is the seventeenth of a series on dietary requirements for reproduction. The previous papers of the series have appeared for the most part in the Journal of Biological Chemistry.

[2] Reference is made by number (italic) to "Literature cited," p. 91.

[3] Report presented before the Biochemical Division of the American Chemical Society at Milwaukee, Sept. 23, 1923.

milk powder (produced from summer milk) 50 per cent; agar-agar, 2; ferric citrate, 0.2; wheat oil, 3; Harris yeast, 1[4]; dextrin, 43.8. This ration contains an abundance of vitamin E. The writer was very much surprised to find that in this ration, which contained no cod-liver oil, two females out of three gave birth to first, second, and third

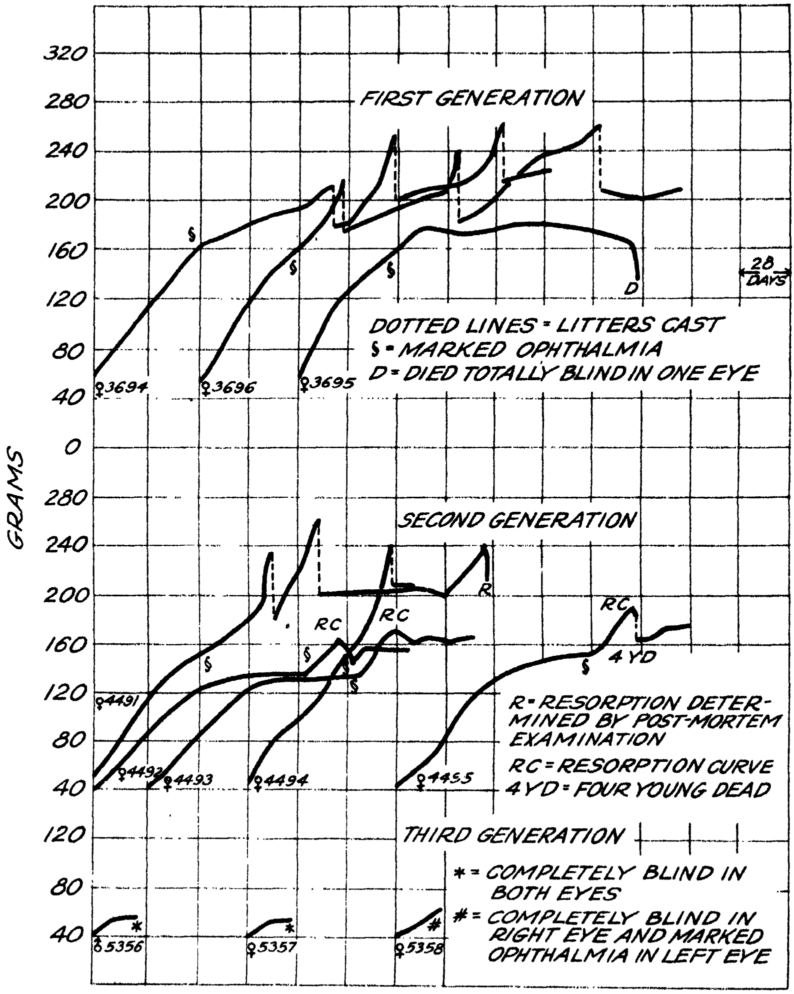

FIG. 1.—Reproduction records of rats on ration 835, adequate in vitamin E but deficient in vitamin A

litters each, and successfully weaned 15 young out of 35 allowed to be reared. These animals must have secured all of their needed vitamins A and D from what they had stored from the previous dietary and what additional amounts they derived from the skim-milk powder and wheat oil. It will be noted, however, that one female (fig. 1) finally succumbed to vitamin A deficiency, having died with pronounced ophthalmia following a rapid loss of body weight. The character of the results on continuous fertility secured with females 3694 and 3696 on ration 835 warranted the continuation of this experiment for succeeding generations. The third litter of female 3694, which consisted of one male and five females, was taken for this purpose.

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[4] From weaning time up to the beginning of the reproduction period the ration contained 0.4 per cent Harris yeast. The increase was made at the time of mating and that percentage left in the ration until the termination of the experiments.

TABLE 1.—*Fertility and lactation records of females on ration 835, adequate vitamin E but deficient in vitamin A*

FIRST GENERATION

Female No.	Litter	Number of young born	Number of young born alive	Number of young allowed to be reared	Number of young weaned	Per cent of young weaned	Remarks
3694	First....	5	5	0	0	0	
	Second..	9	9	6	3	50	
	Third.	6	6	6	0	0	
3695	0	0	0	0	0	0	No signs of advanced pregnancy during the breeding period of 143 days. Died completely blind in one eye.
3696	First....	9	9	6	6	100	Weaned litter in 28 days.
	Second..	9	9	6	0	0	
	Third...	8	8	6	6	100	Weaned litter in 24 days at a normal rate.

SECOND GENERATION (THIRD LITTER OF FEMALE 3696)

Female No.	Litter	Number of young born	Number of young born alive	Number of young allowed to be reared	Number of young weaned	Per cent of young weaned	Remarks
4491	First...	7	7	6	0	0	
	Second..	10	10	6	0	0	
4492	0	0	0	0	0	0	Animal showed typical resorption curve.
4493	0	0	0	0	0	0	Animal showed typical resorption curve. Incipient ophthalmia in both eyes 8 days before termination of experiment.
4494	First...	8	6	6	6	100	Reared first litter in 28 days. Resorbed second litter, as determined by post-mortem examination (fig. 1). At the time of resorption mother showed pronounced ophthalmia in both eyes.
4495	First....	4	0	0	0	0	Prior to the birth of the 4 dead young this female showed a resorption curve.

Figure 1 and Table 1 show clearly that, on the whole, the reproduction record of the second generation was a failure. Although one female had two litters, two animals produced no young, having shown resorption curves[5] indicating that implantation occurred but that the products of conception were resorbed. One female out of the five, No. 4494, had a normal first litter but resorbed the second litter, as determined by post-mortem examination and a study of the uterine horns. Figure 2 shows a photograph of the uterine horns containing the embryos that have undergone a process of resorption for 72 hours.

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[5] A few words of explanation are necessary regarding the meaning of "resorption curves," referred to in the text, in Table 1, and in Figure 1. During the seventeenth to nineteenth day of gestation females manifest unmistakable external signs of advanced pregnancy. At this stage they are placed in individual compartments and are given nests of shavings and excelsior in galvanized boxes. From then on they are weighed daily. When normal fertility proceeds, the mother gains most markedly during the last three to five days of gestation and the birth of the litter is followed by a precipitous drop in weight. When, however, resorption of the embryos occurs the mother instead of gaining markedly during the latter period of pregnancy loses gradually in weight, 6 to 12 gm. in 24 hours and all signs of advanced pregnancy disappear. When such animals are killed the embryos are invariably found in a process of resorption in the uterine horns, and the growth curve shown by such individuals is what the author has previously described as the "resorption curve." Considerable experience in the field justifies the writer in concluding from an analysis of the pregnancy curves and daily observations of the animals that the character of sterility of females 4492 and 4493 was that of resorption.

The character of this picture indicates a condition identical with that manifested by resorption of the fetus during gestation produced by a deficiency of vitamin E demonstrated in a previous communication (*10*). During the stage of resorption this animal showed marked ophthalmia in both eyes, indicating vitamin A deficiency. Female 4493 showed incipient ophthalmia in both eyes during the last eight days of the experiment. The male of this experiment, while showing excellent growth throughout the whole period of the study, suddenly developed an accentuated condition of ophthalmia in both eyes several days before the termination of the investigation. Female 4495 had only one litter of four young which were born dead.

Out of a total of three litters cast only one was successfully weaned (the first litter of female 4494). This litter consisted of four males and two females, but since the purpose of the study was to determine the effect of ration 835 on the reproductive capacity of succeeding generations, the two females and one male only were taken for the third generation. In less than four weeks male 5356 and female 5357 developed ophthalmia so severely in both eyes that they became completely blind, and female 5358 became completely blind in the right eye and showed marked ophthalmia in the left eye. The marked eye lesions were preceded by a cessation in growth in two animals out of the three.

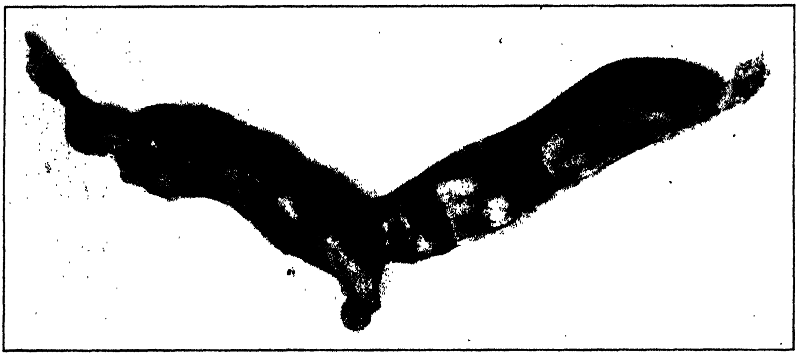

FIG. 2.—Uterine horns of female 4494 containing embryos that have undergone a process of resorption for 72 hours. Photographed by David G. Hall of the Department of Entomology, University of Arkansas

DISCUSSION

The experimental data submitted show clearly that skim-milk powder ration 835, containing 3 per cent wheat oil, furnished considerable amounts of vitamin A for first and second generation animals. That it failed to furnish a sufficient quantity of this vitamin, however, became quite apparent in the second generation and most marked in the third. According to Sherman and MacLeod, skim-milk powder contains 10 per cent as much vitamin A as does whole-milk powder (*5*). The skim-milk powder of ration 835 must, therefore, have furnished some vitamin A, but certainly not enough to have allowed such excellent growth for a long period during the first and second generations. The author is, therefore, obliged to conclude that wheat oil contains considerably more vitamin A than is reported in the literature. Quantitative biological studies on the vitamin A content of wheat oil, the results of which are described in the paper accompanying (*13*) revealed the fact that this oil, the most potent source of vitamin E previously demonstrated by Evans and Bishop (*2*), by the author (*9*), and by Evans and Burr (*3*), contains appreciable amounts of the A vitamin. Such results are at variance with preliminary qualitative data reported during last year (*11*).

In 1925 Sherman and MacLeod (*5*) from their studies of the relation of vitamin A to growth, reproduction, and longevity concluded that diets deficient in vitamin A, but which they claim were satisfactory in vitamin E, impaired fertility. These investigators did not determine, however, the character of the sterility they encountered.

According to Evans and Bishop (*1*), a deficiency of vitamin A produces in females a disturbance of oestrus which is highly characteristic of vitamin A avitaminosis. They find that "it consists in the prolongation of the oestrous desquamative change in the vaginal epithelium, the smear consisting chiefly, if not exclusively, of the cornified cells which in normal individuals characterize the actual period of oestrus and ovulation only, but which, in the case of animals showing vitamine A deficiency, occur throughout the entire period of acute deficiency." From such findings it is quite apparent that when there is an early depletion of vitamin A in the first generation oestrus and ovulation are disturbed and no fertilization is possible; hence, the sterility resembles in character that produced by vitamin B deficiency (*1*). The latter condition is precisely what was recently reported by Parkes and Drummond (*4*) in their study "The Effects of Fat-soluble Vitamin A Deficiency on Reproduction in the Rat." These authors concluded that "the sterility was due, primarily, to physiological debility and disinclination to copulate."

It is quite evident from Table 1 and Figures 1 and 2 that the character of sterility encountered in the present study is specifically associated with resorption of the fetus during gestation. The finding of ophthalmia in the sterile females during periods of resorption of the embryos on a diet abundant in vitamin E warrants the conclusion that the sterility encountered on ration 835 (from which cod-liver oil was absent) was produced by vitamin A deficiency.

SUMMARY

Sterility, characterized by resorption of the fetus during gestation, and associated with vitamin A deficiency, has been produced in rats on a skim-milk powder diet containing an abundance of vitamin E.

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DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION: THE VITAMIN A CONTENT OF WHEAT OIL[1]

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INTRODUCTION

The early work of McCollum, Simmonds, and Pitz on "The Nature of the Dietary Deficiencies of the Wheat Embryo" (*3*)[2] suggested a toxic factor in wheat oil, and possibly for this reason investigators have been discouraged from attempting a quantitative study of the vitamin-A content of this oil. The researches of Osborne and Mendel (*5*) and Voegtlin and Myers (*13*), however, did not disclose a toxic factor in the oil of the wheat embryo. In the reproduction studies of the writer during the past five years more than 12 gallons of wheat oil have been used, and the introduction of as much as 3 per cent of this oil in the diets of rats has not resulted in an impairment of growth, fertility, or lactation, even when the animals were carried as far as the fifth generation (*10*). Recently Simmonds, Becker, and McCollum (*6*) have reported the beneficial effects of wheat oil when added to a "so-called salt ophthalmia-producing ration." The toxicity theory of McCollum and his associates concerning wheat oil promulgated in 1916 can, therefore, be abandoned.

Last year Steenbock and Coward (*7*) developed a quantitative method for the determination of vitamin A, which method insures the provision of vitamin D by irradiating the ration, and with their technic these authors demonstrated that of the three cereals—oats, wheat, and corn—wheat is the most potent in vitamin A. They have not, however, studied the oil of the wheat embryo, which is the source of the fat-soluble vitamins of the wheat kernel.

Since wheat oil is at present used by a number of nutritional investigators as a source of vitamin E, any data concerning its content of additional vitamins should be of interest to these workers, and such information may also serve as a guide in constructing rations deficient in all known fat-soluble vitamins other than E. Simmonds, Becker, and McCollum (*6*) and Mattill (*4*) assume in their recently published papers that wheat oil is quite deficient in vitamin A, and this assumption undoubtedly influenced the interpretation of their experimental findings. In the present communication quantitative data are presented showing that wheat oil, the most potent source of vitamin E, contains appreciable amounts of vitamin A.

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[1] Received for publication May 14, 1928; issued October, 1928. Research paper No. 64, Journal Series University of Arkansas. This report is the eighteenth of a series on dietary requirements for reproduction. (See footnote 1, p. 87.)

[2] Reference is made by number (italic) to "Literature cited," p. 98.

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METHOD OF STUDY

Young nursing albino rats whose maternal diet was deficient in fat-soluble vitamins (*12*) were allowed to remain with the lactating mothers on the same ration for one to two weeks after weaning until unmistakable symptoms of ophthalmia developed, indicating a depletion of vitamin A. The composition of the fat-soluble-deficient diet (No. 1034) used in these experiments was as follows: Casein,[3] 20 per cent; McCollum's salts No. 185, 4 per cent; yeast,[4] 10 per cent; and dextrin, 66 per cent. The animals were from 5 to 6 weeks old at the time the experiments were started. The character of the eye lesions varied in intensity among the different individuals. Only one animal of the entire group showed no signs of ophthalmia at the beginning of the experiment. At the onset of ophthalmia all the animals had shown a complete cessation of growth. The curative method was then adopted, and wheat oil was daily administered separately from the ration to each animal in graduated amounts with a calibrated pipette. As controls, litter mates were employed which received comparable amounts of a cod-liver oil in use in this laboratory since 1921 (*11*). The wheat oil was prepared by percolating whole-wheat embryo with cold acetone by methods previously described (*9*), and was fed in dosages of 0.01 c. c., 0.05 c. c., and 0.1 c. c. per animal per day. The cod-liver oil was fed in dosages of 0.01 c. c., and 0.05 c. c. daily to each rat. Since it was believed that a daily allowance of 0.05 c. c. of cod-liver oil per animal would supply an optimum amount of vitamin A, the 0.1 c. c. cod-liver oil dosage was not tried. The animals were weighed twice a week (and in some cases when necessary three and four times a week) and food-consumption records (Table 1) were taken simultaneously with the records of body weight. The results of the feeding experiments are shown in Figures 1 to 3.

TABLE 1.—*Weekly food-consumption records of ophthalmic rats on a diet containing wheat oil or cod-liver oil as sources of vitamin A*

Animal No.	Number of grams of food consumed during—																								
	1st week	2d week	3d week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week	13th week	14th week	15th week	16th week	17th week	18th week	19th week	20th week	21st week	22d week	23d week	24th week	25th week
4751	[a]10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4752	[a]6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4757	34	38	45	38	38	35	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4758	30	32	31	50	43	33	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4749	47	64	100	100	95	96	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4750	43	62	99	70	73	74	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4763	32	48	63	64	64	40	78	100	93	88	95	99	100	98	102	67	77	85	47	52	53	19	...	...	...
4764	24	43	54	41	56	51	68	79	41	64	65	70	67	69	65	56	65	70	51	68	79	70	69	71	64
4761	50	77	68	95	103	96	90	79	89	78	75	80	80	100	97	81	78	88	80	86	84	65	67	73	70
4762	45	72	81	72	86	85	68	85	81	73	75	79	67	89	83	66	70	76	70	72	67	69	60	63	75
4753	[a]3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4754	30	47	51	66	79	76	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4765	50	75	65	63	46	60	83	103	98	93	94	96	45	100	108	83	85	93	86	92	77	56	...	...	...
4766	47	65	44	52	42	47	75	92	85	68	87	90	55	86	90	65	78	81	67	76	94	65	43	[b]40	...

[a] Food consumption record for a 5-day period. [b] Food consumption record for a 4-day period.

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[3] Purified by repeated extraction with hot 95 per cent alcohol.
[4] Obtained from a commercial yeast manufacturing company.

EXPERIMENTAL RESULTS

It is quite clear from a study of Figure 1 that 0.01 c. c. wheat oil does not begin to compare with 0.01 c. c. cod-liver oil per animal per day as a source of vitamin A. Note, however, the response of animals 4757 and 4758 when taken in the incipient state of ophthalmia. Growth for two to four weeks is quite apparent.

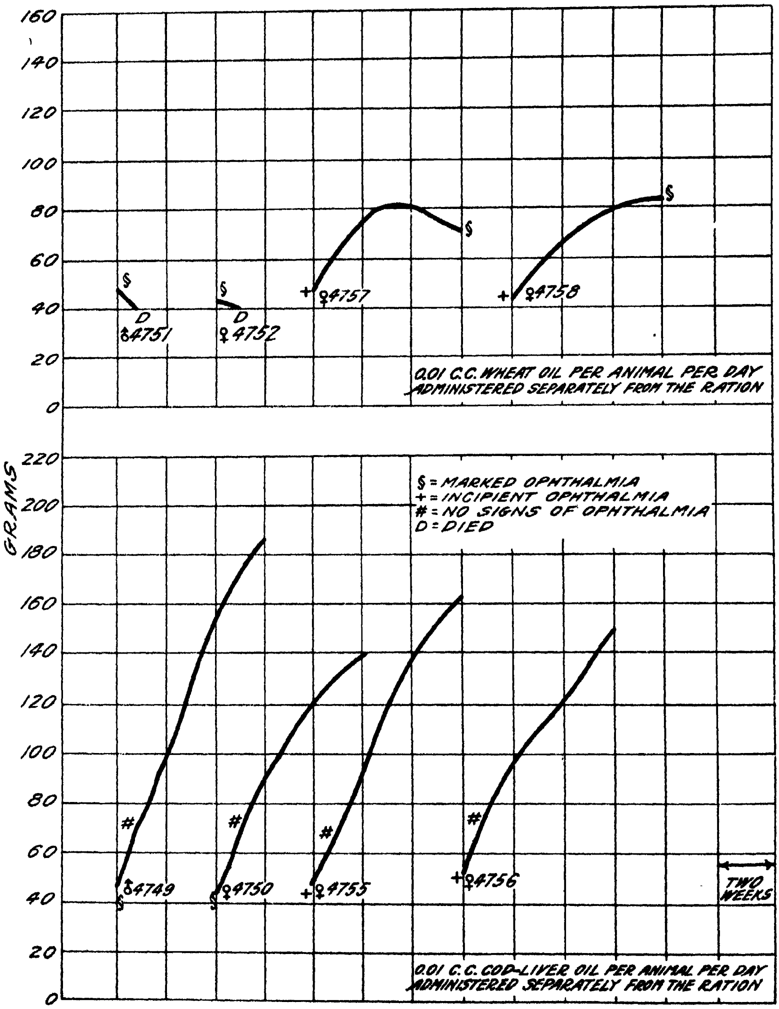

FIG. 1.—Comparative response of ophthalmic rats to vitamin A in wheat oil and in cod-liver oil when fed at low levels

Male 4763 and female 4764 (fig. 2), which at the beginning of the experiment were severely affected by ophthalmia and had already ceased to grow, showed a marked response to the daily administration of 0.05 c. c. wheat oil. Not only did the severe eye lesions entirely clear up in less than four weeks, but excellent growth was obtained in male 4763 for 16 weeks and very good growth in female 4764 for a period of 10 weeks. Animal 4763 finally succumbed, dying totally blind in both eyes when both animals 4761 and 4762

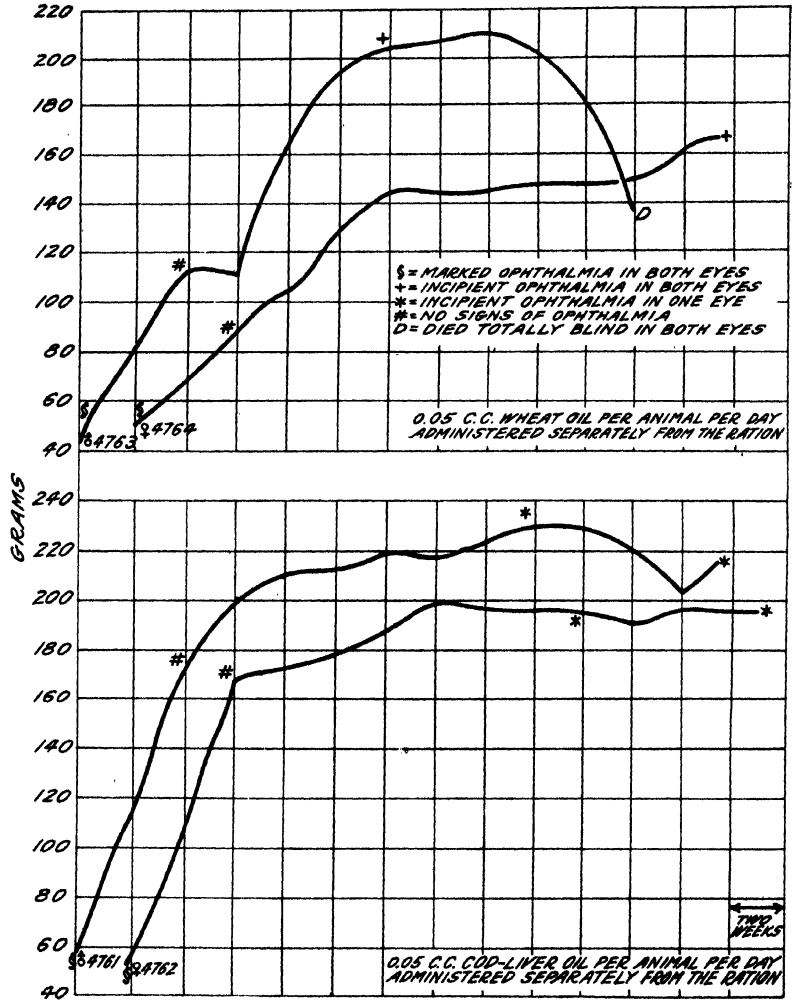

FIG. 2.—Comparative response of ophthalmic rats to vitamin A in wheat oil and in cod-liver oil when fed at levels higher than those represented in Fig. 1. The severity of the eye lesions in animals 4761 and 4762 was much greater than in animals 4763 and 4764. The former animals were almost blind in both eyes when the experiment began. This fact probably explains the longer period required for their eyes to heal

on an equivalent daily allowance of cod-liver oil were in excellent physical condition although showing incipent ophthalmia in one eye. From such results it is concluded that the optimum daily dosage of cod-liver oil for the adult rat is probably nearer 0.1 c. c. than 0.05 c. c. The ophthalmic condition of female 4764 on the 0.05 c. c.

wheat oil daily dosage was no worse than that of the two animals (4761 and 4762) receiving the equivalent cod-liver oil daily allowance, but the growth of No. 4764 was quite inferior. This experiment shows conclusively, then, the appreciable amounts of vitamin A in the oil of the wheat embryo.

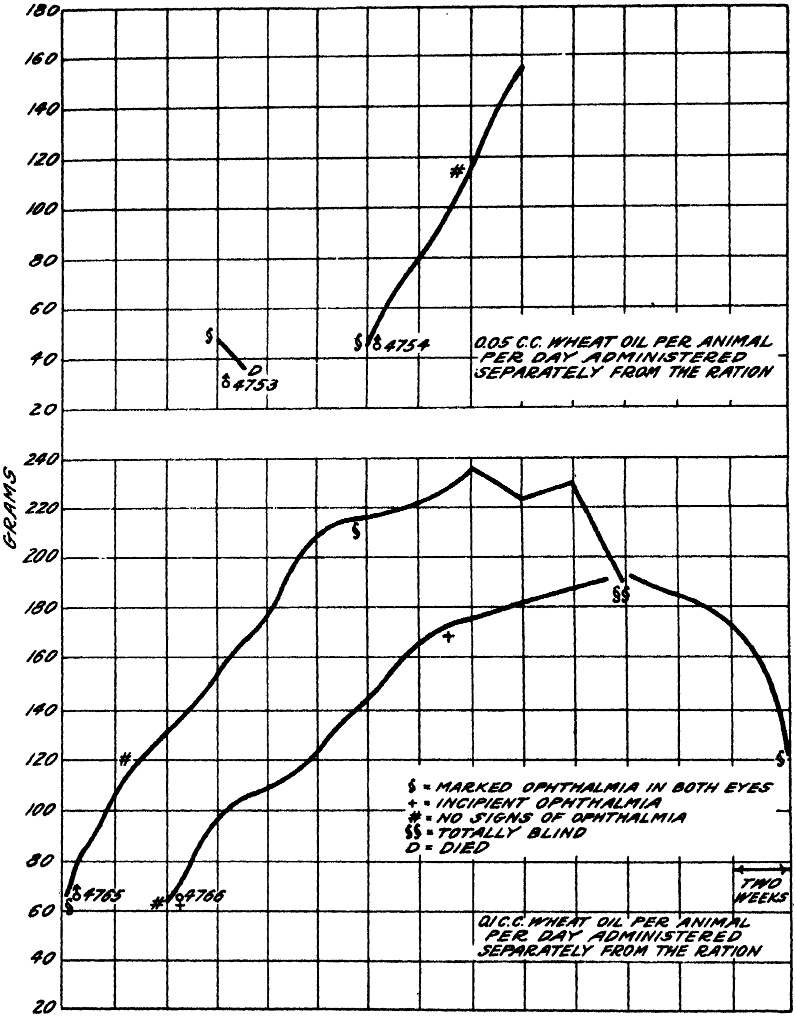

FIG. 3.—Comparative response of ophthalmic rats to vitamin A in wheat oil when fed at high levels

A daily dosage of 0.05 c. c. of wheat oil administered to male 4754 (fig. 3), started on the experiment in the incipient stage of opthalmia and at a period of maintenance of growth, resulted in rapid growth, which was continuous for a period of six weeks. All signs of eye lesions completely disappeared in less than four weeks. The same daily dosage of wheat oil was ineffectual, however, as a therapeutic measure in curing a case of severe ophthalmia (male 4753). A daily dosage of 0.1 c. c. of wheat oil was potent to the extent of entirely clearing up an advanced stage of ophthalmia in 17 days. On such a daily dosage excellent growth was produced for four months, after which period the animals gradually declined in growth and finally collapsed.

Table 1 shows that variations in growth of the individuals can very well be ascribed to variations in food consumption. The point that this table brings out is that before failure on the wheat-oil administrations sets in when the severe ophthalmia manifests itself, showing unmistakably a deficiency of vitamin A, there is a marked reduction of the food intake, until at the very last stage of the avitaminosis complete inanition occurs. Animal 4763 (fig. 2) ate only 1 gm. of food during the last four days of the experiment and animal 4766 ate absolutely nothing during the last three days of the experiment and only 4 gm. during the preceding two days. These findings have considerable significance in the etiology of anorexia, or loss of appetite. For some time it has been known that vitamin B has a controlling effect on food consumption *(1, 2)* and recent work from this laboratory has demonstrated that as little as 5 mgm. of a highly concentrated vitamin B preparation from yeast can bring about a resumption of appetite at a state of complete inanition in 24 hours, but from the work reported in this paper it seems that a deficiency of fat-soluble vitamin A also plays a determining rôle in inanition. To be sure, in this series of experiments ample provision has not been made for the D vitamin, but since this vitamin, according to Steenbock and Nelson *(8)*, is also essential for growth, the animals on ration 1034, deficient in fat-soluble vitamins, must have been deriving considerable vitamin D during the four months of their excellent growth from the supplementary daily administration of wheat oil. It would seem, then, that wheat oil also contains appreciable amounts of vitamin D. Work in progress shows that in cases of uncomplicated vitamin A deficiency, the D vitamin having been provided by the irradiated ration, a gradual reduction in food consumption occurs.

SUMMARY

A dosage of 0.05 c.c. of wheat oil per animal per day furnishes enough vitamin A for excellent growth for a period of 10 to 16 weeks. Such a dosage also serves as a potent therapeutic agent for curing severe eye lesions in animals produced by previous depletion of vitamin A.

The depletion of vitamin A is accompanied by inanition, a symptom heretofore associated only with vitamin B deficiency.

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THE INFLUENCE OF THE SOIL REACTION ON THE IONIZABLE CONSTITUENTS OF THE TOMATO AS DETERMINED BY ELECTRODIALYSIS[1]

By E. S. Haber[2]

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INTRODUCTION

Within recent years electrodialysis as a means of studying certain chemical and physical phenomena has come into use. In studying the effect of soil reaction on the growth of greenhouse plants, especially the tomato, electrodialysis has been used to determine the ratio of acid and basic materials taken up by the plant, depending on the soil reaction (pH value).

A complete review of the literature on electrodialysis up to 1927 is given by Spiegel-Adolph (*11*)[3] and will not be repeated here. Moore, Reeves, and Hixon (*7*) made use of the Mattson (*6*) cell in studying apple tissue affected with Jonathan spot and substantiated Pentzer's (*8*) finding that Jonathan spot is accompanied during storage by a loss of acids in the diseased area.

Bradfield (*2*) by means of the Mattson cell, using KCl, K_2SO_4, and KH_2PO_4, all normal solutions with respect to the potassium content, concluded that in every case the cation was removed more quickly than the anion. The rate of removal of the cation was influenced by the nature of the anion with which it was combined, while the rate of removal of the anions was in the order of $Cl > SO_4 > H_2PO_4$. In every case the cation was removed quantitatively, but the time required was greater, especially in the case of the phosphate ion. Bradfield suggested that the rate of removal of anions would be facilitated by the substitution of a positive membrane for the negative parchment membrane on the anode side.

MATERIALS AND METHODS

New compost soil was placed in the greenhouse bench. The individual plots were separated by boards that extended the entire depth of the bench in order to prevent the soil of one plot from mixing with that of another. The reaction (pH value) of the soil when placed in the benches was 6.5.

Three soil reactions (pH values) were decided upon, one extremely alkaline, pH 8.5–9.0, one neutral or nearly so, pH 6.5–7.0, and one extremely acid, pH 4.0–4.5. These plots were all run in duplicate. To secure the alkaline reaction, the soil was treated with hydrated lime in sufficient quantity so that the pH value was about 9.0 a week after treatment when a fair degree of equilibrium was reached. For the neutral plots the soil was not treated, since it was nearly neutral without treatment. The acid reaction was secured by adding

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[2] The writer wishes to express his thanks to Dr. R. M. Hixon of the Chemistry Department for helpful suggestions and criticisms and for the use of equipment.

[3] Reference is made by number (italic) to "Literature cited," p. 114.

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phosphoric acid (H_3PO_4) so that the reaction was 4.0 a week after treatment.

The tomato plants used were of the Bonny Best variety. The seed was sown in a flat, the seedlings pricked off when 1½ inches high and planted in 2-inch pots, later shifted to 4-inch pots, and finally transferred to the experimental plots 10 days after the soil had been treated. The soil used in potting was ordinary compost with a pH value of 6.0–6.5. The crop was benched October 1 and completed its growth February 1. Determinations of the pH values of the soil of the various plots were made at 10-day intervals after the crop was benched. Small amounts of hydrated lime or phosphoric acid were added from time to time to keep the pH within the desired range.

Electrodialysis of the fruit was made with samples picked from the vines and used immediately. Ionizable constituents of the root, stem, and leaf were determined from air-dried material. The Mattson (*6*) cell for electrodialysis as modified by Clark, Humfeld, and Alben (*4*) was used for this purpose. The procedure was similar to that of Moore, Reeves, and Hixon (*7*) with the exception of size of samples, time intervals, and voltage. In running samples of the root system it was necessary to make a composite sample of two root systems, since 3 gm. of the ground tissue were used and the root system of one plant did not furnish adequate material. In all cases of stems and leaves 3-gm. samples of individual plants were used. The air-dried material was ground in a Wiley mill and passed through a 60-mesh sieve. In the case of the fruits, samples of fresh material composed as nearly as possible of equal sized fruits were made up. Tests were run on both ripe and green fruit. The green fruits were picked just as a faint trace of yellow appeared at the blossom end. By this means it was possible to get composite samples of fruits of about the same degree of maturity. When very green fruits were picked, it was impossible to determine the degree of maturity. Some blossoms were tagged the day they were hand-pollinated, but since all blossoms pollinated on the same day did not mature their fruits anywhere near the same time this gauge of maturity was not reliable. Both ripe and green fruits were run through a food chopper until they were of a creamy consistency. It was impossible to attain this condition with very green fruits, which was another reason for using the fruit just as the first trace of color appeared at the blossom end. Fruits used for analysis when just showing a faint trace of color at the blossom end will be referred to henceforth as "green mature."

In case of the ground tissue of the plant 3 gm. were placed in 300 c. c. of distilled water in the middle compartment and 275 c. c. of water in each of the anode and cathode compartments. The parchment paper used to separate the compartments was dialyzed one hour in distilled water to remove ionizable constituents. The parchment paper was kept in a humid atmosphere before it wasused in the cell, for unless this was done the swelling was so great after the water was added as to cause the papers to become limp and flaccid.

Electrodialysis of fruit, leaf, stem, and root tissue rendered none of the pigments soluble, so no discoloration of parchment paper occurred. Consequently, the same parchment paper was used until broken or torn. If the paper was stretched tightly the samples checked much more closely. After the sample had been dialyzed the compartments were filled with distilled water and the paper was redialyzed to remove any ions remaining from the previous sample; 250 gm. of the green fruit or 275 gm. of the ripe fruit, after being made up to volume of 300 c. c. with distilled water in a 500 c. c. graduate, were placed in the middle compartment, and 275 c. c. of distilled water were used in each of the cathode and anode compartments; 105 to 110 volts of direct current from storage batteries were passed through the cell for all the root, stem, and leaf samples. For the electrodialysis of the fruits 82 volts were used for the first four periods and 108 volts for the others.

All data given are the results of three to five analyses on samples from each plot. These checked within narrow limits when conditions were as nearly alike as possible. In order to obtain comparable samples the platinum electrodes must always occupy the same position in the cell and the parchment membranes must be stretched tight and not allowed to bulge. When these precautions were observed it was relatively easy to obtain checks within 2 to 4 c. c. where the total N/10 titrable acid or base would run as high or higher than 200 c. c. The material which came out on the basic side (anolyte) was titrated with N/10 H_2SO_4 and on the acid side (catholyte) with N/10 NaOH. At the end of the designated time the anolyte and catholyte were drained and the current was shut off. It required about two minutes to drain and refill the anode and cathode compartments, and then the current was turned on again and the time was recorded from the starting and stoppage of the flow of current. This method differed from that of Moore, Reeves, and Hixon (7) in that they left the current flowing while draining and refilling to prevent a return of the ions in the middle compartment. However, closer checks were obtainable where the current was shut off and the same length of time used each period for draining and refilling. Titrations on the acid side (catholyte) were made with N/10 NaOH, but are given in the results as the amount of titratable acid present in the plant tissue. Titratable base determined with N/10 H_2SO_4 are given in terms of N/10 base present in the tissue.

EXPERIMENTAL DATA

Table 1 includes some physical and chemical measurements of ionizable material in the ripe fruits. The voltage given is the initial voltage recorded at the beginning of the period of dialysis. A small drop in voltage occurred where the amperage ran above 1.0, but at no time was the drop more than 5 volts by the end of the period. The dialyzate was drawn off frequently enough to prevent the temperature from going above 30° C. Titratable acid and base are given in cubic centimeters and are figured on a basis of N/10.

Differences in acid and basic constituents due to soil reaction were very small in the ripe fruit. Within 5 hours and 20 minutes all of the basic constituents were removed from fruits taken from plants grown on the alkaline plots and within 4 hours from the neutral and acid plots. There were 4.48 per cent more N/10 base present in the samples from the plots with alkaline reaction than in those from the neutral plots. Conversely, there was 4.87 per cent less N/10 base in the fruits from the acid plots than in fruits from the neutral plot. Fruits from plants grown on the acid soil contained very little more acid than fruits grown on the highly alkaline soil.

TABLE 1.—*Results of electrodialysis of ripe tomato fruit from soils of different pH values*

Time	Current	Soil pH 8.5-9.0			Soil pH 6.5-7.0			Soil pH 4.0-4.5		
		Charge	Base	Acid	Charge	Base	Acid	Charge	Base	Acid
Minutes	*Volts*	*Amperes*	*C. c.*	*C. c.*	*Amperes*	*C. c.*	*C. c.*	*Amperes*	*C. c.*	*C. c.*
10	82	1.30	19.2	10.2	1.50	25.4	12.8	1.40	23.4	10.5
10	82	1.30	20.2	10.2	1.10	20.4	11.7	1.30	22.4	10.6
20	82	3.40	86.6	29.9	3.05	82.4	28.6	3.50	81.4	30.1
20	82	1.15	37.9	17.3	1.05	39.0	17.7	1.10	39.0	18.1
20	108	0.85	21.4	15.8	0.80	15.0	14.6	0.90	15.0	16.5
40	108	1.10	14.2	21.2	0.80	10.8	18.2	0.80	10.8	21.9
40	108	1.00	10.3	18.3	0.40	8.7	15.6	0.20	1.7	18.9
80	108	0.55	3.7	26.1	0.65	3.7	27.0	0.30	1.7	28.1
80	108	0.25	1.1	17.9	0.25	0.0	20.7	0.10	0.0	15.6
80	108	0.15	0.0	14.3	0.10	0.0	17.5	0.10	0.0	18.8
400	....	....	214.6	181.2	....	205.4	184.4	....	195.4	189.1

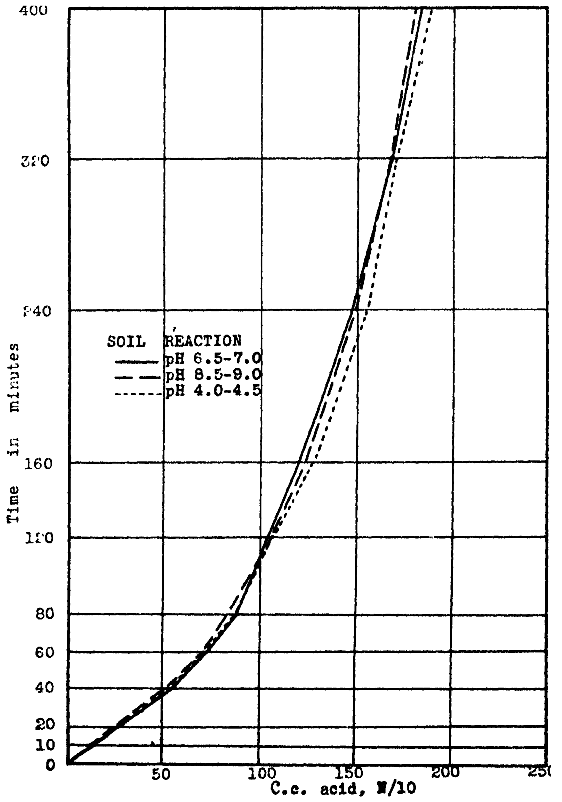

FIG. 1.--The rate of removal of acids by electrodialysis from the ripe fruit of tomato plants grown on soils of different hydrogen-ion concentration

On the other hand, the acid constituents were removed much more slowly than the basic ones. Again, differences due to soil reaction were noted, but they were very small and probably not significant, since all of the acid constituents were not removed in the time allowed for electrodialysis. The fruit from plants on the alkaline plots contained 1.73 per cent less acid than fruits from the plants on the neutral plots, but fruit from the acid plots contained 2.54 per cent more acid than fruits from the neutral plots.

The acid constituents were removed at a more uniform rate than the basic constituents, as shown in Figures 1 and 2.

Positive correlation between the soil reaction and ionizable acids and bases of the green mature fruit is shown in Figures 3 and 4.

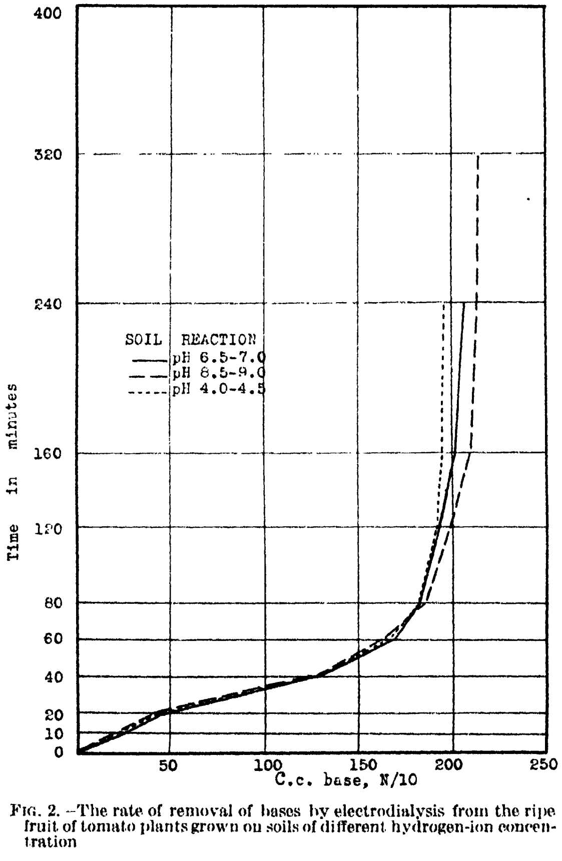

FIG. 2.—The rate of removal of bases by electrodialysis from the ripe fruit of tomato plants grown on soils of different hydrogen-ion concentration

Differences in the ionizable acids and bases in the green mature fruit, depending on the soil reaction, were similar to those found in the ripe fruit. Basic constituents were 2.4 per cent greater in fruits from plants of the alkaline plots and 4.95 per cent less in the fruits from plants of the acid plots as compared with those from the neutral plots. There was 5.01 per cent less acid in fruits from the alkaline plot and 8.37 per cent more acid in the fruits from the acid plots as compared with those from the neutral plots. The percentage differences were greater for acids in the green mature fruit from the acid and alkaline plots compared to the neutral plots than in the case of the ripe fruits.

Bases were removed from the tissue at a much faster rate than the acids, and although the rate of removal of the acids was much slower it was at a more uniform rate, as shown in Figures 3 and 4. Since the acid constituents were removed much more slowly than the basic ones, electrodialysis of the green mature fruits was carried on for approximately 27 hours to determine if the relative differences of acid or basic constituents, depending on the soil reaction, were changed by electrodialysis over a longer period. The relative differences between the rate of removal of basic and acid constituents as shown in Figures 5 and 6 were practically the same as those given in Figures 3 and 4 for the green mature fruit from the neutral and acid plots when electrodialysis was carried on for the short period.

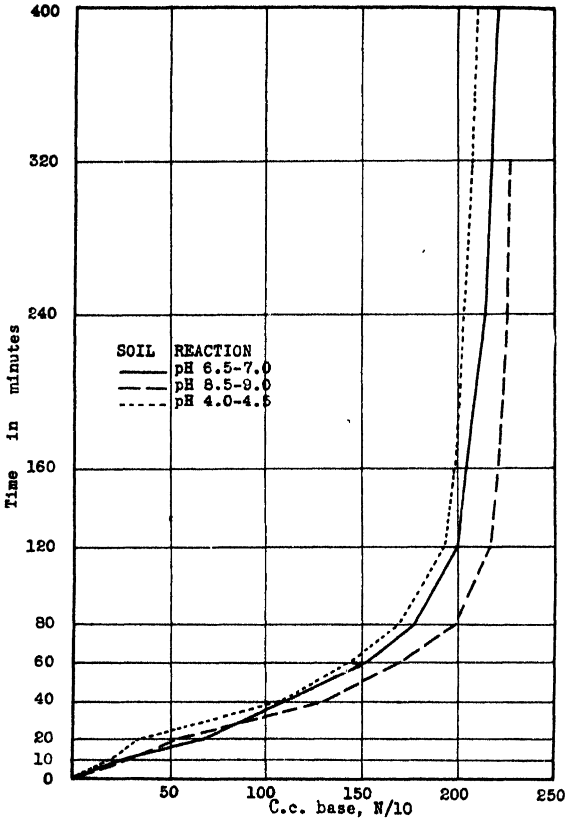

FIG. 3.—The rate of removal of bases by electrodialysis from the green fruit of tomato plants grown on soils of different hydrogen-ion concentration

The bases were removed in 5 hours and 20 minutes from the neutral plots and in 4 hours from the acid plots. This corresponds to the length of time required for the removal of bases, as shown in Figure 3, in which case electrodialysis was carried on for 6 hours and 40 minutes. Although the acids were not completely removed at the end of the long period, there was a difference of 7.34 per cent between the fruits from the neutral and aklaline plots as compared with a difference of 8.37 per cent for the short period. The rates of removal of acids and bases are shown graphically in Figures 5 and 6.

A comparison of the ionizable constituents of the leaves from the three series of soil reactions showed a tendency similar to that of the fruit. The leaves from the plants from the plots with an acid reaction contained more acid and less base than leaves from plants from the neutral plots, while leaves from plants grown on soil with an alkaline reaction contained less acid and more base than leaves from plants grown in neutral soil. The rate of removal of acid and base is shown in Figures 7 and 8.

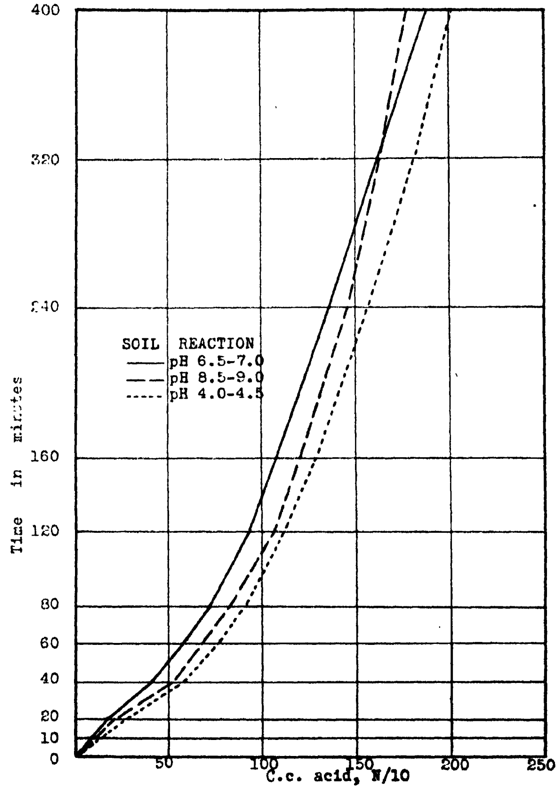

FIG. 4.—The rate of removal of acids by electrodialysis from the green fruit of tomato plants grown on soils of different hydrogen-ion concentration

The pH values of anolyte and catholyte of the leaf material were made by means of the Bailey (*1*) hydrogen electrode. The E. M. F. readings were converted to pH values from the tables given by Schmidt and Hoagland (*10*). Differences in titratable acid of the catholyte between some of the runs from the same tissue were very great, but the relative change in pH was very slight. The range in pH from the catholyte from all the plots was from 2.33 to 3.07. Changes in pH from the anolyte were greater, showing a range from 11.59 to 8.74. Differences in pH corresponded to the differences in titratable base, but the relative differences in the case of the pH values were not as great.

Data graphically presented in Figures 9 and 10 indicate that the ionizable constituents of the stems were influenced by the soil reaction. Percentage differences in titratable N/10 acid and base, depending on the soil reaction in which the plants were grown, were greater than with leaves and fruit. Differences in pH values of the catholyte were small compared to the amount of titratable acid. Changes in pH of the anolyte showed a greater positive correlation with changes in titratable base.

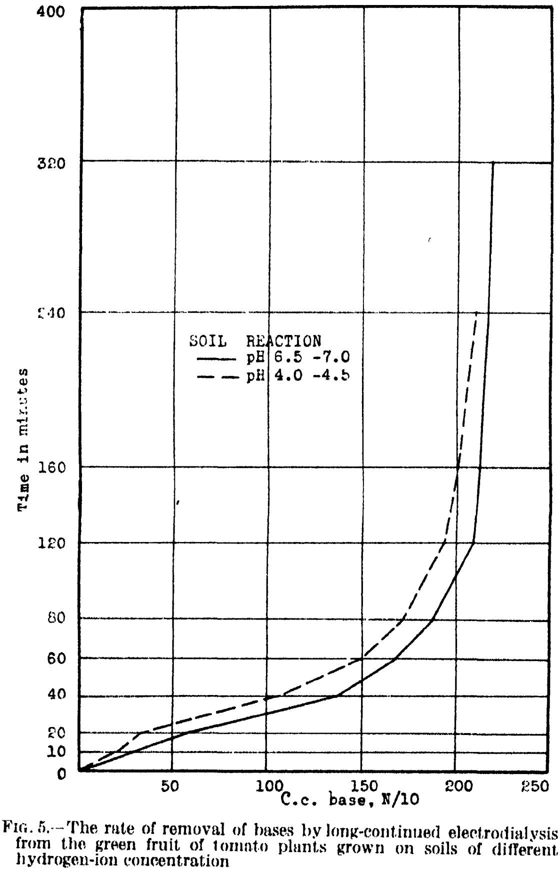

FIG. 5.—The rate of removal of bases by long-continued electrodialysis from the green fruit of tomato plants grown on soils of different hydrogen-ion concentration

The greatest differences between amounts of ionizable acid and base, depending on the soil reaction, were found in the root tissue. Root tissue from the alkaline plots contained 78.06 per cent more N/10 base than like tissue from the neutral plots. However, the tissue from the acid plots contained only 15.19 per cent less base. The root tissue contained 60.14 per cent more N/10 acid when the plants were grown in an acid soil than when they were grown in a neutral soil. Changes in pH of the catholyte were small; however, differences in the titratable acid were great. Changes in pH of the anolyte corresponded to changes in titratable base to a greater degree than with any of the other tissues dialyzed. Figures 11 and 12 indicate the rate at which the N/10 acid and base were removed.

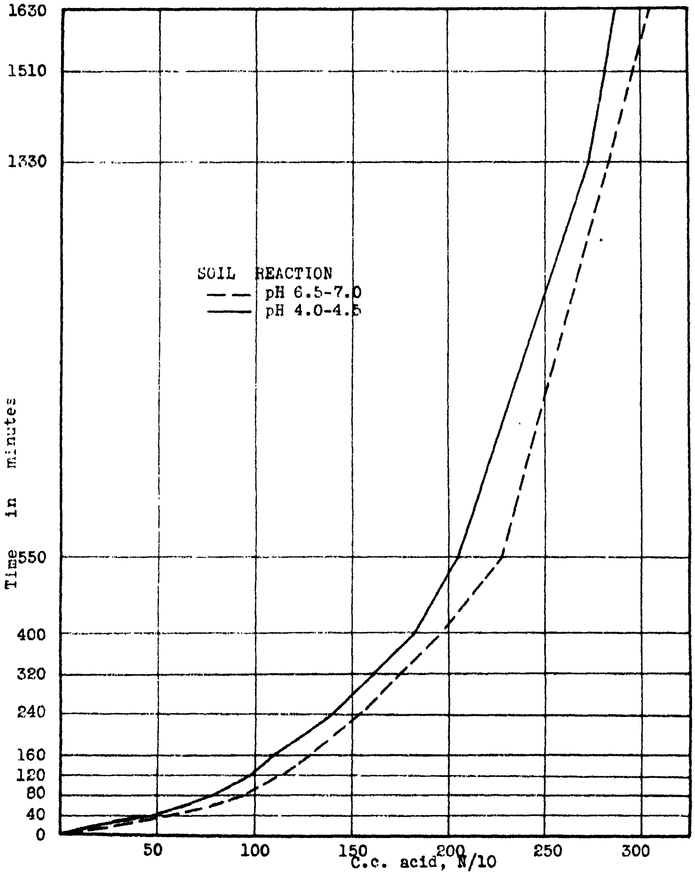

FIG. 6.—The rate of removal of acids by long-continued electrodialysis from the green fruit of tomato plants grown on soils of different hydrogen-ion concentration

Table 2 is a brief summary of the more important findings. Differences in total titratable N/10 acid and base only are included. Differences in cubic centimeters and percentages of the amount of acid and base contained in the respective tissues are presented. Findings with respect to the amount of ionizable constituents in tissues from plants grown in the neutral plots were taken as a basis of comparison.

TABLE 2.—*Difference in titratable acid and base from various parts of the tomato plant*

Sample	Soil reaction	N/10 base	Difference		N/10 acid	Difference	
		Cubic centimeters	*Cubic centimeters*	*Per cent*	*Cubic centimeters*	*Cubic centimeters*	*Per cent*
Ripe fruit	pH 8.5-9.0	214.6	+9.2	+4.48	181.2	−3.2	−1.73
	pH 6.5-7.0	205.4			184.4		
	pH 4.0-4.5	195.4	−10.0	−4.87	189.1	+4.7	+2.54
Green mature fruit	pH 8.5-9.0	225.5	+5.3	+2.40	178.1	−9.4	−5.01
	pH 6.5-7.0	220.2			187.5		
	pH 4.0-4.5	209.3	−10.9	−4.95	203.2	+15.7	+8.37
Leaves	pH 8.5-9.0	192.6	+10.7	+5.93	104.0	−5.9	−5.46
	pH 6.5-7.0	181.9			109.8		
	pH 4.0-4.5	175.6	−6.3	−3.46	121.1	+21.2	+19.29
Stems	pH 8.5-9.0	126.9	+23.2	+22.37	82.5	−10.0	−10.81
	pH 6.5-7.0	103.7			92.5		
	pH 4.0-4.5	91.5	−12.2	−11.76	112.0	+19.5	+21.08
Roots	pH 8.5-9.0	103.1	+45.2	+78.06	44.7	−10.7	−19.56
	pH 6.5-7.0	57.9			54.7		
	pH 4.0-4.5	49.1	−8.8	−15.19	87.6	+32.9	+60.14

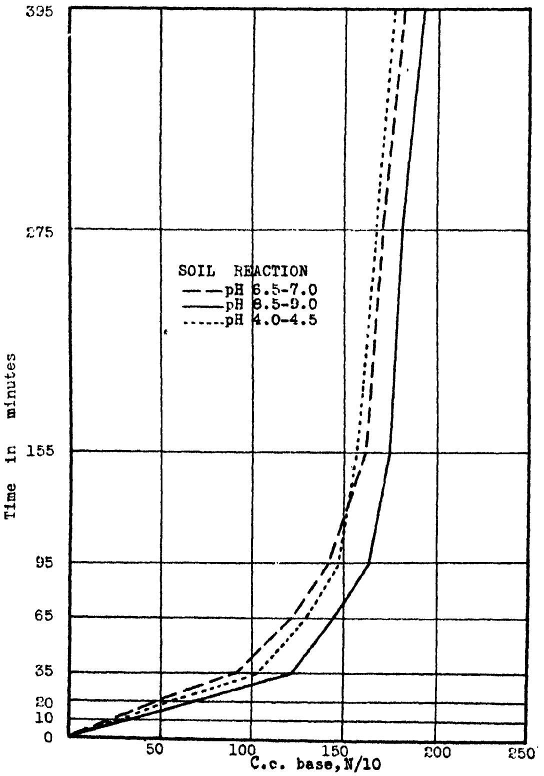

FIG. 7.—The rate of removal of bases by electrodialysis from the leaves of tomato plants grown on soils of different hydrogen-ion concentration

DISCUSSION

Three grams of air-dried tissue was used in all cases where roots, stems, and leaves were dialyzed. Fresh material was used with the fruit, 250 gm. of the "green mature" and 275 gm. of the ripe fruit.

According to Wehmer (*13, p. 678*), the ripe tomato fruit contains 92 to 94 per cent of water. Therefore the fruit samples contained 7 to 9 gm. on a dry-weight basis. This was two to three times as much as the root, stem, and leaf samples. Consequently, the roots and fruits contained the least amount of ionizable material and the leaves contained the greatest amount, as measured in terms of N/10 titratable acid and base.

The various samples of the plant tissue used were as large as practicable in order to obtain a large amount of acid and basic material. Since the titrations were with N/10 H_2SO_4 and N/10 NaOH, the larger the total amount of acid or base required to neutralize the dialysate the smaller the error. The size of the sample was governed by the amperage in a given length of time. It was not deemed advisable to allow the amperage to go above 4 for more than one or two minutes, and it was preferable to hold it below 2, since with high amperage high temperatures resulted. It may be noted that the amperage corresponded to the amount of acid and base removed, especially in the early stages of dialysis. The greater the total of

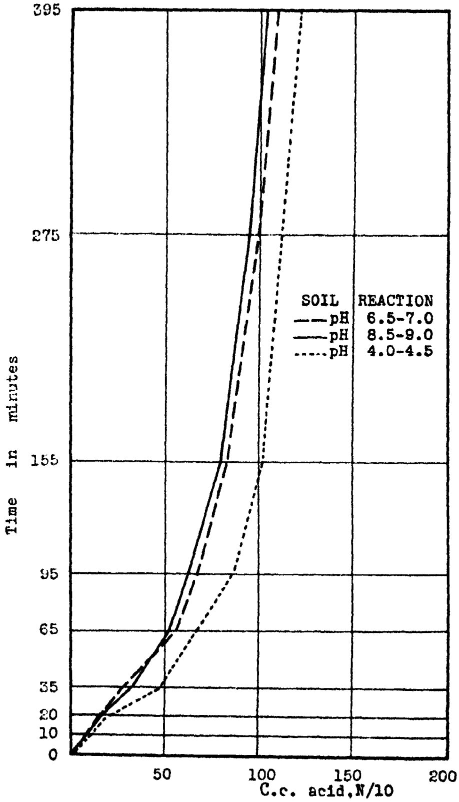

FIG. 8.—The rate of removal of acids by electrodialysis from the leaves of tomato plants grown on soils of different hydrogen-ion concentration

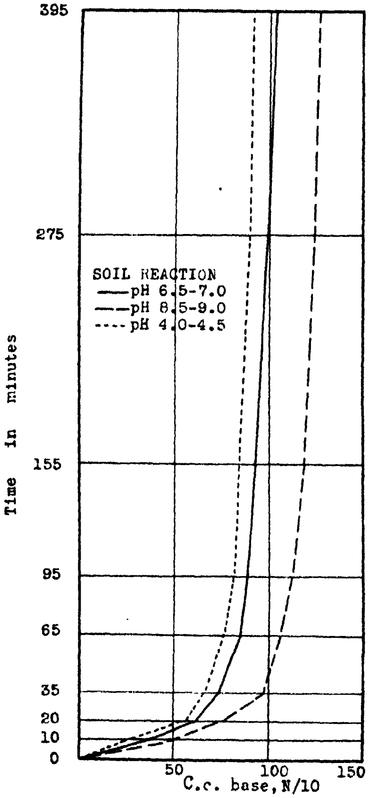

FIG. 9.—The rate of removal of bases by electrodialysis from the stems of tomato plants grown on soils of different hydrogen-ion concentration

ionizable constituents present in the dialysate, the greater the amperage.

The greatest percentage differences of the acid and basic constituents, depending on the acidity or alkalinity of the soil, were found in the root systems. These differences decreased in the order of roots, stems, leaves, green mature fruit, and ripe fruit.

It is not surprising to find the greatest differences in acid and basic materials in the root systems of plants grown in acid and basic soils. Since the alkaline soil was treated with hydrated lime, one would expect to find a greater amount of calcium salts present in the plants. The acid soil having been treated with phosphoric acid would contain more acid material. Hartwell (*5*) found the precentage of phosphorus in turnips to be positively correlated with the amount of available phosphorus in the soil. Truog (*12*) pointed out that the pH value of the tissue or extract of a number of agricultural plants could be raised by the addition of lime to acid soils in which the plants were growing. This would indicate the intake of a greater amount or proportion of alkaline material. The findings of Reed and Haas

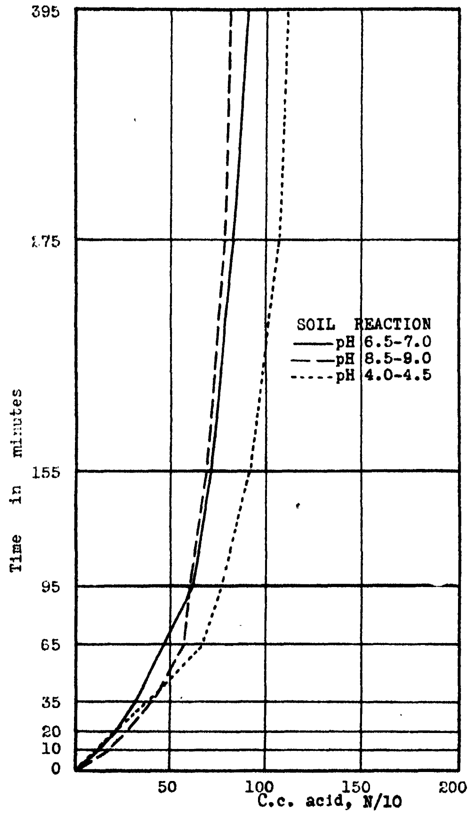

FIG. 10.—The rate of removal of acids by electrodialysis from the stems of tomato plants grown no soils of different hydrogen-ion concentration

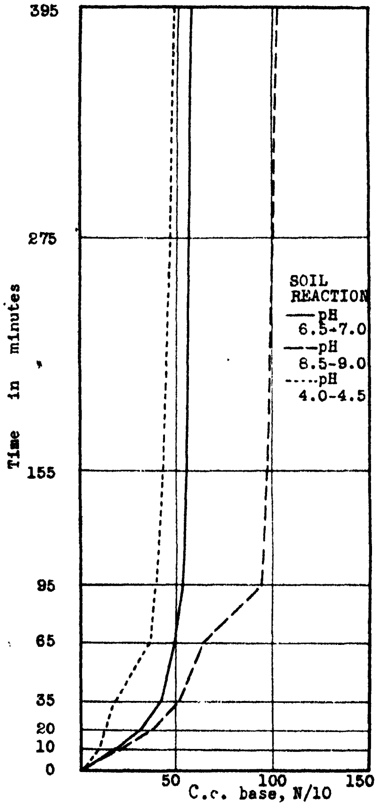

FIG. 11.—The rate of removal of bases by electrodialysis from the roots of tomato plants grown on soils of different hydrogen-ion concentration

(*9*) were contrary to this, as they reported no differences in pH values of the sap expressed from walnut seedlings regardless of the reaction of the solution in which they were grown.

As stated previously, the differences between titratable acid and base, depending on the acidity or alkalinity of the soil, became less in the order of root, stems, leaves, and fruit. Burgess and Pember (*3*) found the highest percentages of aluminum in the roots, considerable in the leaves, a little in the stems, and none in the grain of barley. It may be logical to suggest from their results and from those reported in these experiments that there is an equalization of base and acid materials by the time the ionizable material reaches the fruit.

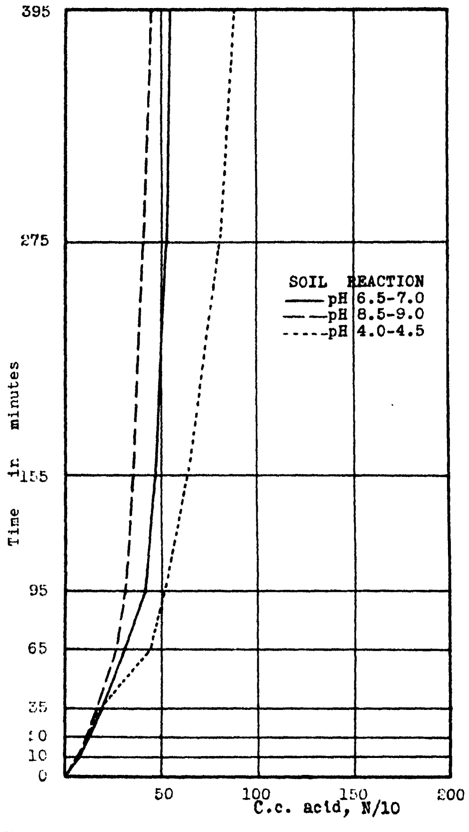

FIG. 12.—The rate of removal of acids by electrodialysis from the roots of tomato plants grown on soils of different hydrogen-ion concentration

The pH values of anolyte and catholyte were taken for roots, stems, and leaves, but the differences were small, especially where the amount of titratable acid and base was large. This might well be expected where the solutions were well buffered.

SUMMARY

More acid materials as determined by electrodialysis were found in plants grown on acid soil and more basic materials in plants grown on alkaline soil. The percentage differences were in the order root, stem, leaf, and fruit.

The leaves contained the greatest amount of ionizable materials; roots and fruit apparently contained the least.

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RAPID GROWTH OF CHICKS ON RATIONS OF NATURAL FOODSTUFFS [1]

By ALBERT G. HOGAN, CHARLES L. SHREWSBURY, and HARRY L. KEMPSTER, *Missouri Agricultural Experiment Station*

INTRODUCTION

During the course of studies on the nutritional requirements of the chick, diets of natural foodstuffs were frequently used as controls for the synthetic rations. Surprising variations were noted, however, in the rate at which different individuals grew, even on rations of commercial materials, which were presumably adequate in every respect. It finally became apparent that the variability was due to unsuspected deficiencies in the rations employed, and an attempt was made to formulate a more satisfactory diet. This effort [2] was successful beyond expectation, for not only was the variability decreased, but the rate of growth was more rapid than any recorded, as far as the writers are aware, for the particular breed of chicks used in these experiments. However, these earlier successes were without immediate interest except for experimental purposes, because of the high price of the ration. An attempt was therefore made to prepare from more readily available constituents a ration that would be equally effective in supporting growth.

EXPERIMENTAL PROCEDURE AND RESULTS

During the last two years a large number of rations were tested in an effort to develop one giving some promise of practical value. However, only four of these are described herein and, as is evident from Table 1, all of them are very similar in composition, although corn predominates in two of the rations and wheat in the other two.

TABLE 1.—*Composition of experimental rations*

Components of ration	Percentage composition of ration—			
	No. 564	No. 803	No. 806	No. 810
	Per cent	*Per cent*	*Per cent*	*Per cent*
Yellow corn	65.5		65.5	
Wheat		65.5		65.5
Liver meal	15.0	15.0		
Tankage			15.0	15.0
Dried buttermilk	10.0	10.0	10.0	10.0
Alfalfa meal	5.0	5.0	5.0	5.0
Cod-liver oil	2.0	2.0	2.0	2.0
Sodium chloride	1.0	1.0	1.0	1.0
Calcium carbonate	1.5	1.5	1.5	1.5

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[1] Received for publication July 5, 1928; issued October, 1928.

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Key No. Mo.-10

Only White Leghorn chicks have been used, and all were reared under strictly laboratory conditions, as described in an earlier publication.[3] The solids of the ration were intimately mixed, in a finely divided condition, and the cod-liver oil was then added. Fresh mixtures were made up each week, or oftener. The all-mash system of feeding was followed exclusively, chiefly for convenience. No attempt was made to determine whether or not it is superior to other methods that are more commonly used.

The first ration used, No. 564, proved fairly satisfactory, but the chicks' rate of growth was considerably below the optimum. A more successful ration, described in an earlier paper,[4] contained dried whole milk, and it seemed to the writers that the inclusion of milk would probably make ration 564 equally satisfactory. The use of whole milk is impractical, but skim milk offered some promise as a substitute. Water, therefore, was withdrawn entirely from some groups of chicks and skim milk was offered instead.

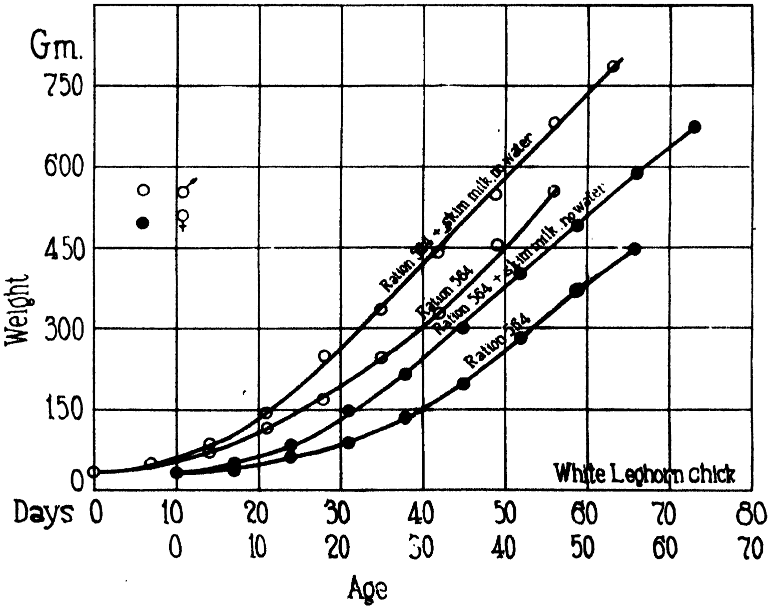

FIG. 1.—Growth of chicks as affected by skim milk in the ration. All chicks were given the same basal ration, No. 564. Those that received skim milk in addition grew more rapidly than those that received water

In all, three groups of chicks, at different times, have been reared on ration 564, and they have all grown at about the same rate. Four groups have received skim milk in addition to ration 564, and they too have grown very uniformly. In Figure 1 are presented the growth curves of groups on these rations, kept under observation at the same time, and therefore strictly comparable.

A number of modifications of ration 564 have been tried that seem worthy of mention, though detailed description will not be attempted.

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[3] HOGAN, A. G., GUERRANT, A. B., and KEMPSTER, H. L. CONCERNING THE ADEQUACY OF SYNTHETIC DIETS FOR THE GROWTH OF THE CHICKS. Jour. Biol. Chem. 64: 113–124, illus. 1925.

[4] HOGAN, A. G., HUNTER, J. E., and KEMPSTER, H. L. Op. cit.

At three different times ration 672, a mixture of 90 parts of ration 564 and 10 parts of dried yeast, was used, with a considerable degree of success. The mixture supported growth at practically the same rate as ration 564 and skim milk. Growth was a little more rapid still when skim milk was fed along with ration 672, though the difference was not great.

The modifications of ration 564 that were of most interest involved the substitution of tankage for liver meal and of wheat for yellow corn. The combination of corn and tankage has been definitely inferior to any of the others, and as a rule rations containing corn are slightly inferior to those containing wheat, but the inferiority is slight except when tankage is used as a protein concentrate. It was surprising to note that the wheat-tankage combination was practically as effective as any other used. A comparison of growth rates on the four combinations tried is presented in Figure 2.

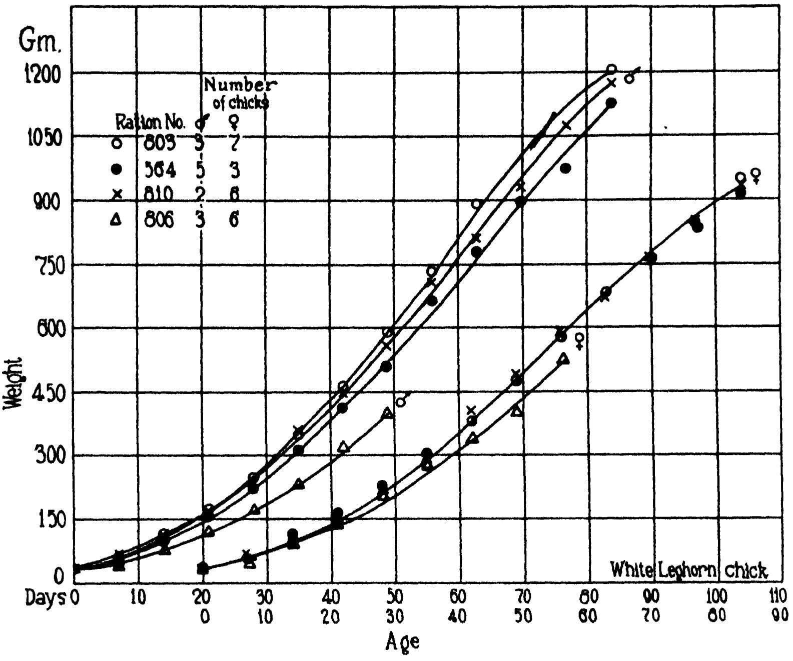

FIG. 2.—Growth of chicks on various rations. The composition of the rations is given in Table 1. Of the combinations listed, corn and tankage is least effective. Rations containing wheat are superior to those containing corn

In order to present a more complete view of the growth of these chicks a graph (fig. 3) is included which shows the growth of a group that was kept under observation until it reached maturity. For purposes of comparison an age-weight curve of chicks which were grown by standard methods is also included. So far as the writers are aware these controls made more rapid growth [5] than any others of the same breed reported in the literature.

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[5] BUCKNER, G. D., WILKINS, R. H., and KASTLE, J. H. THE NORMAL GROWTH OF WHITE LEGHORN CHICKENS. Amer. Jour. Physiol. 47: 393–398, illus. 1918.

An inspection of Figure 3 shows that chicks on ration 803 supplemented with skim milk grew more rapidly and attained a mature weight at an earlier age than the controls. Whether this difference is due to the ration or to genetic factors can not be decided with the evidence available. It may be that the strain of chicks used is smaller and attains maturity more quickly.

In connection with the rate of growth, the initial weights of the chicks may be significant. The average weight at hatching of the males described herein was 31 gm., and the average weight of the females was 33 gm. In contrast with these, the average weight of the males used as controls was 42 gm. and the average weight of the females was practically 41 gm. The chicks with the heavier initial weights usually grow most rapidly.

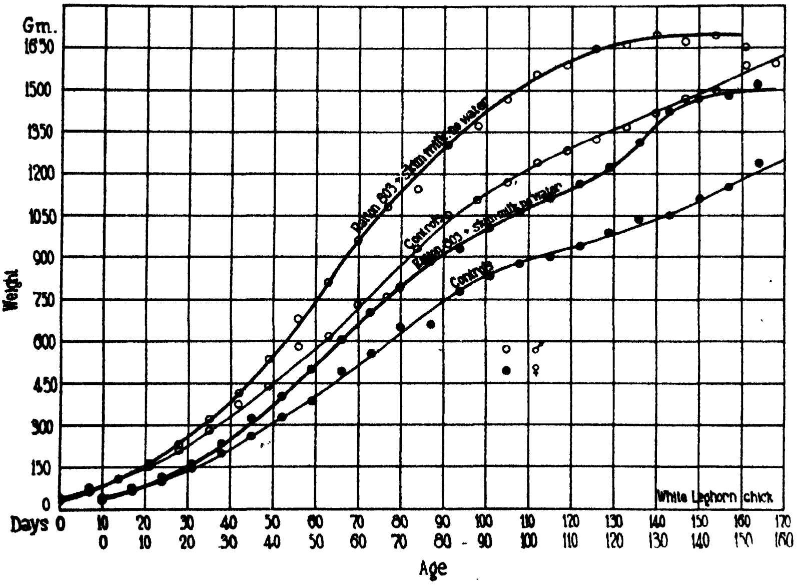

FIG. 3.—Growth of chicks to maturity on a wheat ration supplemented with skim milk. These are age-weight curves of one of the few groups of chicks that were permitted to attain mature weights. The chicks used as controls were, so far as is known, the most rapidly growing yet described

One difficulty, the incidence of leg weakness, encountered in rearing chicks by the method used in these experiments requires a brief description. In all 146 chicks were reared on the rations described and on various modifications, and of these 16, or practically 11 per cent, developed a form of leg weakness characterized chiefly by gross deformities. This abnormality can not be explained from what is now known of avian physiology. Data reserved for future publication show that the bones are normal in composition and that the percentages of calcium and of inorganic phosphorus in the blood are normal. It seems certain that the condition is not rickets, and there is no definite indication of polyneuritis. Sporadic attempts have been made to prevent this abnormality by using various mineral mixtures, but to no avail. A few chicks were growing poorly when the symptoms were noted, though deaths were rare. In some instances the most rapidly growing chicks had badly deformed legs. Experiments are now under way to decide whether crowding and close confinement may be responsible. Typical illustrations of this type of leg weakness appear in Figure 4, A, B.

Before leaving the question of leg weakness entirely, an instance should be described that merits recording, though the abnormality may not be identical with the one described in the preceding paragraph. In an earlier paper [6] ration No. 400 was mentioned as being used with variable success. This ration contains whole wheat, 65.5; whole milk powder, 9.7; commercial casein, 14.5; alfalfa meal, 2.9; milk fat, 4.9; sodium chloride, 1.0; and calcium carbonate, 1.5.

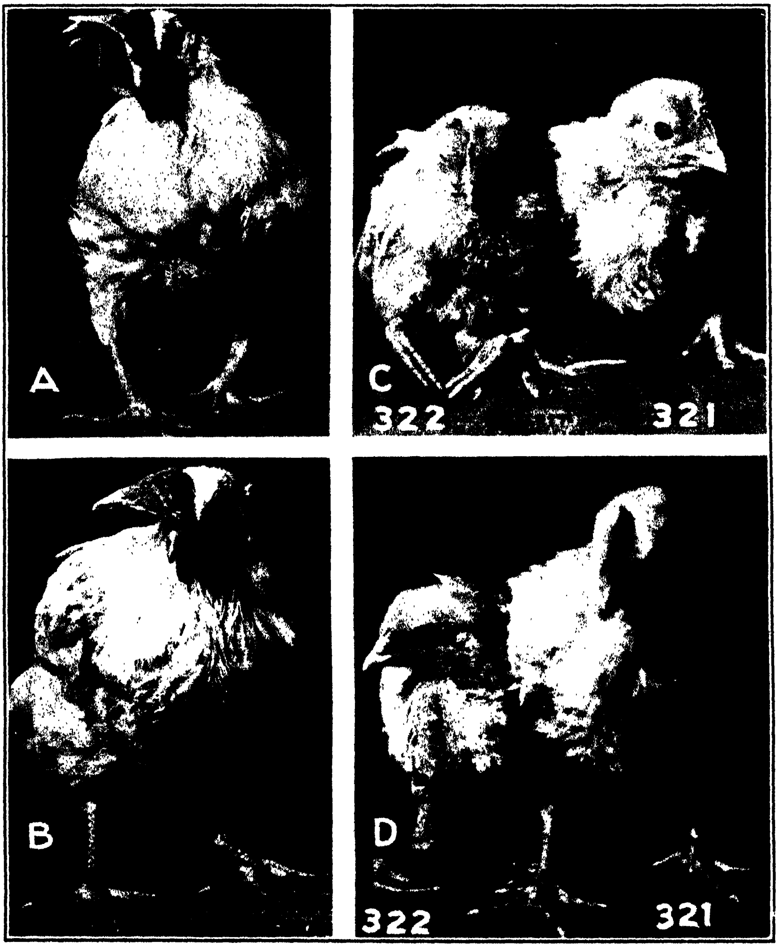

FIG. 4.—A, B.—These chicks illustrate the type of leg weakness encountered so frequently in the experiments herein reported. The one shown in B is most typical. Treatment with yeast, in small quantities at least, is ineffective. C.—Two chicks which developed leg weakness at 25 days of age and were unable to stand. D.—The chicks shown in C, but greatly improved by treatment with yeast. They ultimately recovered. When this photograph was taken they were 27 days old

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[6] HOGAN, A. G., HUNTER, J. E., and KEMPSTER, H. L. Op. cit.

One morning, 25 days after a group of chicks had been placed on this ration, two of them were found in an almost helpless condition. One was given considerable quantities of yeast and appeared almost normal two days later. The other was given a small quantity of yeast and cod-liver oil in addition and made less improvement by the second day. Larger quantities of yeast were then given, and on the fourth day both were normal in appearance. One had a relapse on the eithteenth day after the first attack, but treatment with yeast over a seven-day period seemed to bring about complete recovery.

It appears improbable that the response of the two chicks just described to ration 400 can be adequately explained on the basis of knowledge at present available. It seems certain, however, that the diet is only partially adequate, and that the inadqeuacy can be remedied by feeding yeast. Presumably the deficient substance is intimately related to vitamin B. Figure 4, C, D, illustrates the appearance of these chicks at the time of collapse and after treatment with yeast.

The mortalities are of considerable importance, so a report of these losses is included herein. In addition to the 146 chicks that were reared, there were 2 that died in their sixth week, another was killed because it had become badly hampered by deformities of the legs, and another was killed by accident. Besides these 4, there were 30 others that died or were killed within the first two weeks. Practically all of these were diagnosed as suffering from diarrhea, but how many cases were of the bacillary type can not be said. Most of these chicks were purchased from a commercial hatchery, so it is possible that they were infected when brought to the laboratory. The mortality rate would, of course, be more significant if it were known how many chicks were infected at the beginning of the observations.

The records of food consumption are also of interest, though chicks are wasteful feeders, and accuracy is not easily attained. One can only be sure that not more than a certain amount of food was consumed. The records were obtained on groups of chicks, and each group contained individuals of both sexes. The records are summarized in Table 2.

It is evident that if comparable stages of the growing periods are considered, chicks compare very favorably with other animals in the economy of growth. During the first two weeks less than 2 pounds of feed were required to produce a gain of 1 pound. At the end of the sixth week the weight of the males was practically 1 pound, and the food consumption per pound gain had not yet reached 3 pounds. Following the sixth week the cost of gains continued to increase until by the end of the twelfth week 6 or 7 pounds of food were required to produce a gain of 1 pound. A rough calculation shows that when a chick weighs 1 pound it has consumed about 3 pounds of food. About 8 pounds are required to being a chick to a weight of 2 pounds, and 13.5 pounds of food are necessary to bring it to a weight of 3 pounds.

The commercial value of the rations herein described may be regarded as promising, but tests under practical conditions have not yet been completed. If it should seem profitable to use extensively rations of the type described in this paper, further study should be given to their composition, and to the methods of using them. It is entirely possible that the formulas could be so changed as to make them less expensive without decreasing their usefulness. Such changes unquestionably could be made in the later stages of the growing period.

TABLE 2.—*Gain in weight and food consumption of growing chicks on different rations*

Age of chicks	Ration No. 803					Ration No. 561				
	Gain in weight per week	Weight of dry food	Weight of skim milk	Total solids consumed [a]	Solids consumed per gram of gain in weight	Gain in weight per week	Weight of dry food	Weight of skim milk	Total solids consumed [a]	Solids consumed per gram of gain in weight
Weeks	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	242	580	1,390	719	3.0	251	290	1,400	430	1.7
2	461	590	2,325	823	1.8	459	620	2,325	853	1.9
3	491	1,290	3,000	1,590	3.2	525	930	3,000	1,230	2.3
4	686	1,120	4,100	1,530	2.2	444	1,110	4,000	1,510	3.4
5	785	2,250	6,800	2,930	3.7	1,020	710	5,400	1,250	1.2
6	1,080	2,180	8,400	3,020	2.8	895	1,810	7,800	2 590	2.9
7	1,015	2,980	10,000	3,980	3.8	910	3,300	8,000	4.100	4.5
8	1,165	5,570	10,800	6,650	5.7	780	3,570	9,800	4,550	5.9
9	1,190	5,260	14,200	6,680	5.6	1,090	5,510	13,800	6,890	6.3
10	780	4,290	14.200	5,710	7.3	920	3,740	13,400	5,080	5.5
11	905	4,240	14.000	5,640	6.2	700	3,700	14,000	5,100	7.3
12	1,070	4,410	14,600	5,870	5.5	1,150	5,440	14,600	6,900	6.0
Total	9,900	34,760	103,815	45,142	4.6	9,144	30,730	97,525	40,483	4.4

Age of chicks	Ration No. 810					Ration No. 806				
	Gain in weight per week	Weight of dry food	Weight of skim milk	Total solids consumed [a]	Solids consumed per gram of gain in weight	Gain in weight per week	Weight of dry food	Weight of skim milk	Total solids consumed [a]	Solids consumed per gram of gain in weight
Weeks	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	204	190	1,425	333	1.6	164	150	1,400	290	1.8
2	401	560	2,325	793	2.0	325	540	2,200	760	2.3
3	475	690	3.000	990	2.1	408	550	2,900	840	2.1
4	304	960	3,900	1,350	4.4	524	900	3,600	1,260	2.4
5	705	1,800	5,200	2,320	3.3	600	1,890	5,400	2 430	4.1
6	870	1,860	7,800	2,640	3.0	675	2,120	7,000	2,820	4.2
7	710	1,760	8,600	2,620	3.7	610	1,760	7,800	2,540	4.2
8	920	2,500	9,300	3,430	3.7					
9	675	4,920	14,200	6,340	9.4					
10	825	4,730	12,600	5,990	7.3					
11	695	3,750	14,000	5,150	7.4					
12	810	3,790	14,000	5,190	6.4					
Total	7,594	27,510	96,360	37,146	4.9	3,306	7,910	30,300	10,940	3.3

[a] This value is obtained by assuming that the skim milk contains 10 per cent of solids, and adding this calculated value to the weight of dry food consumed. No correction is made for the moisture content of this dry food.

SUMMARY

Rations have been prepared which permit chicks, under laboratory conditions, to grow at an unusually rapid rate. The constituents of these rations are natural foodstuffs, or readily available commercial preparations.

Approximately 10 per cent of the chicks reared in restricted quarters developed leg weakness. This abnormality has not been associated with any known dietary deficiency.

The quantity of food required to produce gains in weight in chickens was similar to that required by other animals at comparable stages of growth.

PHOSPHORUS DISTRIBUTION IN GRAINS [1]

By J. E. WEBSTER [2]

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INTRODUCTION

In past years considerable attention has been given to the subject of nitrogen and carbohydrate fractionation of plant materials, and a vast sum of data has accumulated. Phosphorus and its compounds in plants have, however, been little studied. Recent advances in methods of study, especially of the phospholipin fraction, have directed attention to the phosphorus compounds in plants, and the writer has undertaken to make a rather complete study of changes occurring both in seeds and in green plants. As a preliminary step toward this end, a study of the phosphorus fractions in a group of seeds has been made and the results are reported in this article. Previous workers have made studies on certain of these fractions in grains, but none seems to have made a comprehensive fractionation.

REVIEW OF LITERATURE

Averill and King (*2*)[3] have determined the phytin content of several foodstuffs and give a discussion of the method and modifications they used. Their results are expressed as phytin. Rather (*8*) also has worked on the Heubner and Stadler method for phytin estimation and his procedure is the one followed in the present work. He reports some results for total and acid-soluble phosphorus as well as phytin, in plant materials. Collison (*3*) reports on the inorganic phosphorus content of several grains and also gives an improved method for its estimation, which method is the one used in this work.

The solubility and distribution of phosphorus compounds in seeds has also been studied by Koehler (*7*). His extensive work deals mainly with total, inorganic, and organic compounds in a few seeds, and a large part of his work is on methods.

Guerrant (*5*) has analyzed seeds of various kinds for their phospholipin content and discusses the relation of this fraction to various other constituents such as ash, fat, and protein. He finds no significant relationship. Other workers have made various phosphorus determinations on plant material, but the foregoing summarizes the more important recent publications.

EXPERIMENTAL METHODS AND DATA

The seeds used in the work here reported were all of the 1927 crop and were stored in half-gallon jars with p-dichlorobenzene added as a disinfectant. Openings, screened with fine copper gauze, were made in the caps to permit gas exchange. The seeds, before analysis, were so ground as to pass the finest mesh screen of the Wiley mill.

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[1] Received for publication May 31, 1928; issued October, 1928. Published with the permission of the Director of the Oklahoma Experiment Station.

[2] The writer wishes to express his thanks to the members of the Agronomy Department for the seeds which they furnished and to Dr. Heller for his aid and suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 125.

Two-gram samples of the finely ground seeds were dried overnight at 105° C. and weighed for moisture determination, after which the samples were ashed in a muffle furnace and weighed to secure the ash. The fat percentage was obtained by the usual ether-extraction method run on dried samples.

Germination was made early in the fall and was intended to give some idea of viability, since it was thought that changes in viability might occasion certain phosphorus changes.

The determination of total phosphorus was made according to the official volumetric method (*1*) on samples digested, using H_2SO_4, K_2SO_4, and $CuSO_4$, as was done by Jones and Perkins (*6*).

Phospholipin phosphorus was determined by the microcolorometric method of Guerrant (*4*), modified only to the extent of using 50 c. c. of the alcohol-ether mixture per gram of material in place of the 25 c. c. which he recommends.

The determination of phytin phosphorus was carried out by the iron-titrametric method suggested by Rather (*8*).

Collison's improved method as given in the Journal of Biological Chemistry (*3*) was used without change for the determination of inorganic phosphorus.

The values of other phosphorus were secured by subtracting the sum of the various fractions from the total phosphorus figure. It necessarily follows that this figure is not of great value since it contains the errors of the other figures.

The figures in Table 1 are calculated to a dry-weight basis. In every case a sufficient number of determinations was made to secure closely checking and consistent figures, and wherever possible these were compared with published results in other articles.

TABLE 1.—*Analysis and percentage germination of seeds as related to the distribution of phosphorus*

Seeds analyzed	Percentage germination	Percentage of water	Analysis expressed in percentage of dry weight						
			Fat	Ash	Total phosphorus	Phytin phosphorus	Lipoid phosphorus	Inorganic phosphorus	Other phosphorus
Mung bean	100	8.94	0.77	3.77	0.5305	0.3418	0.0404	0.0239	**0.1244**
Soy bean	100	6.65	16.90	5.54	.5469	([a])	.0966	.0178	--------
Cowpea	95	8.84	1.73	3.28	.4736	.1438	.0459	.0282	**.2557**
Wheat	80	8.68	2.33	1.77	.4274	.3033	.0283	.0210	**.0748**
Barley	72	8.08	2.39	2.96	.3937	.1794	.0220	.0222	**.1701**
Common kafir	62	11.04	3.23	1.82	.3704	.2800	.0206	.0157	**.0541**
Yellow corn	98	8.48	5.25	1.50	.3468	.2865	.0352	.0124	**.0127**
Oats	98	8.23	4.94	3.99	.3453	.1905	.0290	.0149	**.1109**
White corn	94	8.79	5.85	1.48	.3194	.2443	.0485	.0156	**.0110**
Darso	64	10.65	3.57	1.49	.3117	.2714	.0227	.0158	**.0018**
Rye	75	8.79	1.99	1.87	.2947	.2059	.0325	.0197	**.0366**

[a] The solutions were so colored that no satisfactory results could be secured.

DISCUSSION

In discussing the data presented in Table 1, only one salient fact stands out: There seems to be no relation between the total phosphorus content of the seeds and that of any of the fractions, nor is there any comparable variation found among the different fractions. It was thought that perhaps there would be some relation shown between the fat and phospholipin content, but such is not the case. While it is true that low germination appears to be associated with low phospholipin content, this is probably accidental. It was also thought that there might be some relation between the ash content and the inorganic phosphorus, but no constant relationship is apparent. Phytin phosphorus is, in nearly every case, the most important fraction; in only two instances does it constitute less than 50 per cent of the total and in Darso it represents 87 per cent. The inorganic phosphorus represents only a very small percentage of the total, as does also the phospholipin fraction in most instances.

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HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

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INTRODUCTION

The continual increase in prevalence of tobacco ring spot in Virginia after it was first observed in 1917, together with the severe damage that it often caused to the tobacco crop, showed it to be a disease of great economic potentiality These facts led to a study of the disease, the results of which were published in a preliminary report by Fromme, Wingard, and Priode[3] in 1927 In this report ring-spot was shown to be an infectious disease whose causative agent is apparently a virus

The further study of the disease, reported in this paper, relates to its occurrence and symptoms on a variety of hosts other than tobacco, and on a number of species and varieties of Nicotiana

REVIEW OF LITERATURE

In 1922 Fromme and Wingard,[4] and in 1924 Wingard and Godkin reported ring-spot infection on some of the agronomic varieties of *Nicotiana tabacum* L in Virginia Such reports have also been made by the Plant Disease Survey from Kentucky and Ohio In 1927, Fromme, Wingard, and Priode[6] reported four additional species of Nicotiana as being susceptible to infection, namely, *glutinosa, langsdorffii, paniculata* and *sylvestris* and the following varieties of *N tabacum* *Atropurpurea, auriculata, brasiliensis, calyciflora, colossea, gigantea, lacerata, latissima, macrophylla*, and *microphylla* The agronomic varieties, Burley, Green's Wildfire Resistant, Little Orinoco, Macedonian, and Maryland, were also shown to be susceptible

Priode,[7] in 1928, reported ring-spot infection on beet (*Beta vulgaris* L), pokeweed (*Phytolacca decandra* L), petunia (*Petunia hybrida* Vilm), and New Zealand spinach (*Tetragonia expansa* Murr)

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[1] Received for publication June 4 1928 issued October 1928 Paper No 71 from the Department of Botany and Plant Pathology Virginia Agricultural Experiment Station

[2] The writer wishes to acknowledge his indebtedness to Dr F D Fromme for many valuable suggestions in this study and in the preparation of the manuscript For the seed of the different species and varieties of Nicotiana used in the experiments the writer is especially indebted to Dr P J Anderson of the Connecticut Agricultural Experiment Station Dr R E Clausen of the University of California Dr James Johnson, of the University of Wisconsin and Dr W D Valleau of the University of Kentucky

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MATERIALS AND METHODS

The inoculum used throughout these studies was taken from a tobacco plant of the variety Kentucky Yellow obtained in Charlotte County, Va., August 20, 1926. The plant showed typical symptoms of ring spot on all of its leaves and as a result was considerably dwarfed. It was transplanted into the greenhouse bed at Blacksburg, where it continued to grow for over a year.

The ring-spot symptoms gradually failed to develop on the newly formed leaves, but the sap from the new growth continued to be infectious and would readily produce the disease on healthy plants. Infection was obtained on plants of *Nicotiana langsdorffii* Weinm. in December, 1926, and from then on transfers were made at regular intervals to healthy plants of *N. langsdorffii*, and *N. tabacum* in order to keep an abundant supply of disease on young vigorous plants.

Seed of many of the plants other than tobacco were obtained locally from growers and seedsmen, and many of the seeds were collected locally as seedlings and transplanted into the greenhouse beds. The plants in the majority of cases were grown in 4-inch pots which were sunk to about one-third of their depth in the soil of the greenhouse bed. In other cases, however, they were grown directly in the greenhouse bed soil. Plants, like tobacco, that make an erect growth were inoculated when they were 6 to 12 inches high.

The number of plants employed to represent the different hosts in the inoculation experiments varied from only 5 or 6 in a few cases to as many as 50 in others. In most cases the inoculations were repeated several times and at different stages in the development of the plant, new plants being used for each experiment, to make sure that the symptoms produced were typical. Material from 26 of the species of plants listed as hosts has been used as reinoculum on tobacco (*Nicotiana tabacum* L.), and typical ring-spot symptoms have developed in every instance. The species from which inoculum has been recovered and used to reinfect tobacco are indicated by an asterisk in the list of hosts given below. No attempt was made to obtain infection on tobacco from any of the other species, but there is no reason to believe that infection would not have been obtained on tobacco from these species had the inoculations been made.

The inoculum employed has consisted uniformly of the fresh sap and macerated tissues of heavily infected tobacco leaves, prepared by grinding the affected leaves in a mortar and adding enough tap or distilled water to dilute the sap to about 1 to 10. The inoculum was always applied immediately after being prepared in order to make sure of its virulency which disappears rapidly in expressed sap.

Infection has been transmitted readily by several methods of inoculation, but the method found to be most satisfactory and the one employed in this study is designated as the swabbing method. By this method the leaves of the trial plants are carefully swabbed with cotton plugs saturated with inoculum prepared as described above. Three or four leaves intermediate in size and age were inoculated on each plant. The inoculations were made late in the afternoon to avoid scorching of the leaves by the hot rays of the sun. No bell jars or covers of any kind were employed.

EXPERIMENTAL RESULTS

A wide selection of plants was inoculated in order to determine as far as possible the limits of the host range, and to study such variation in symptoms as might occur on different hosts. Especial attention was given to those plants which might be expected to carry the virus over winter in or near tobacco fields.

Infection was obtained on 38 genera of plants representing 17 families; namely, Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Dipsaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Phytolaccaceae, Polygonaceae, Scrophulariaceae, Solanaceae, and Violaceae. The genera, species, and varieties of these families are listed below.

SOLANACEAE.—This family is well represented in the list of hosts, *Nicotiana acuminata* Grah., *N. clevelandii**[8] A. Gray, *N. glutinosa** L., *N. langsdorffii** Weinm., *N. longiflora* Cav., *N. paniculata* L., *N. plumbaginifolia** Viv., *N. quadrivalvis* Pursh, *N. quadrivalvis* var. *multivalvis* Gray, *N. repanda** W., *N. rustica* L. var. Iowa, var. English, and var. *jamaicensis*, *N. sanderae** (*Alta grandiflora* × *forgetiana*), *N. suaveolens* Lehm., *N. sylvestris** Speg., *N. tomentosa* Ruiz. and Pav., *N. trigonophylla* Dun., and *N. tabacum* L. developed infection. The following botanical varieties of *N. tabacum* also developed infection: *Atropurpurea*, *auriculata*, *brasiliensis*, *calycina*, *calyciflora*, *cavala*, *colossea*, *gigantea*, *lacerata*, *latissima*, *macrophylla*, *microphylla*, *purpurea*, *sanguinea*, and Turkish; and also the agronomic varieties, Adcock, Big Burley, Green's Wildfire Resistant, Kentucky Yellow, Little Orinoco, Lizard Tail, Macedonian, Maryland, Standup Burley, and Warne.

Other solanaceous plants found to be susceptible were *Datura stramonium** (Jimson weed), *Nicandra physalodes* (L.) Pers. (apple of Peru), *Petunia violacea** Lindl. (petunia), *Physalis angulata* L. (ground cherry), *Solanum carolinense** L. (horse nettle), *Solanum melongena* L. var. *esculentum* Nees (eggplant), *Solanum nigrum* L. (nightshade), and *Solanum pseudo-capsicum* L. (Jerusalem cherry).

CUCURBITACEAE.—Infection was readily obtained on the following representatives of this family: *Citrullus vulgaris* Schrad. (Monte Cristo watermelon), *Cucumis melo* L. var. *cantalupensis** Naud. (Honey Ball and Honey Dew cantaloupe), *Cucumis sativus** L. (Everbearing and Ideal White Spine cucumber), *Cucurbita pepo* L. var. *condensa** Bailey (Golden Summer Crookneck and Mammoth White Bush squash), *Cucurbita moschata** Duschene (Cushaw pumpkin), *Cucurbita pepo* L. (cornfield pumpkin), *Cucurbita pepo* L. var. *ovifera** Bailey (Nest Egg gourd), *Lagenaria leucantha* Rusby (Dipper gourd), and *Luffa cylindrica** Roem. (Dish Cloth gourd).

COMPOSITAE.—The list of ring-spot hosts in this family includes both weeds and cultivated plants, namely, *Ambrosia artemisiifolia* L. (small ragweed), *Ambrosia trifida* L. (giant ragweed), *Aster laevis* L. (Ostrich Plume aster), *Bidens discoidea* (T. and G.) Britton (Spanish needle), *Calendula officinalis** L. (pot marigold), *Callistephus chinensis* Nees (China aster), *Erigeron canadensis* L. (field erigeron), *Helianthus annuus* L. (Mammoth Russian sunflower), *Lactuca sativa* L. var. *capitata** L. (head lettuce), *Lactuca scariola* L. (prickly lettuce),

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[8] Infection has been obtained on tobacco with an inoculum taken from the species that are marked with an asterisk.

Tagetes erecta L. (African marigold), and *Zinnia elegans** Jacq. (garden zinnia).

LEGUMINOSAE.—A number of species in this family have been shown to be susceptible to ring-spot infection, namely, *Dolichos lablab* L. (Broad Windsor bean), *Melilotus officinalis** Lam. (sweet clover), *Phaseolus lunatus* L. (small Lima or Sieva bean), *Phaseolus vulgaris** L. (kidney bean), and *Vigna sinensis* Endl. (black-eye cowpea).

The families listed below are represented by one or more host species as indicated.

CHENOPODIACEAE.—*Beta vulgaris** L. (garden beet and Silesian sugar beet), *Beta vulgaris* L. var. *cicla* L. (Swiss chard), *Chenopodium album* L. (lamb's-quarters).

VIOLACEAE.—*Viola papilionacea* Pursh (common violet), and *Viola tricolor* L. (giant pansy).

AIZOACEAE.—*Tetragonia expansa* Murr. (New Zealand spinach).

AMARANTHACEAE.—*Amaranthus paniculata* L. (pigweed).

CONVOLVULACEAE.—*Ipomoea purpurea* (L.) Roth. (morning-glory).

CRUCIFERAE.—*Barbarea Barbarea** (L.) MacM. (winter cress).

DIPSACEAE.—*Scabiosa atropurpurea* L. (sweet scabious).

EUPHORBIACEAE.—*Ricinus communis* L. (castor-oil plant).

LABIATAE.—*Salvia splendens* Ker. (scarlet sage).

MALVACEAE.—*Hibiscus esculentus* L. (okra).

PHYTOLACCACEAE.—*Phytolacca decandra** L. (pokeweed).

POLYGONACEAE.—*Polygonum hydropiper* L. (smartweed).

SCROPHULARIACEAE.—*Antirrhinum majus** L. (snapdragon).

In reading the list of host plants given above one is likely to conclude that the ring-spot virus is not very specific in its ability to infect. Such, however, does not seem to be the case, since many plants that failed to develop symptoms of any kind have been inoculated in these experiments. Of course, some of these may prove to be susceptible to infection when subjected to further experimentation, but in that event the number should be small.

No infection was obtained on the following plants: *Althaea rosea* Cav. (hollyhock), *Berberis vulgaris* L. (barberry), *Brassica alba* Rabenh. (mustard), *Brassica napobrassica* Mill. (rutabaga), *Brassica rapa* L. (turnip), *Brassica oleracea* L. var. *capitata* L. (cabbage), *Brassica oleracea* L. var. *acephala* DC. (collards), *Bryophyllum pinnatum* Kurz. (bryophyllum), *Capsicum frutescens* L. var. *grossum* Bailey (bell pepper), *Centranthus ruber* DC. (Jupiter's beard), *Chrysanthemum carinatum* L. (chrysanthemum), *Cirsium arvense* (L.) Scop. (Canada thistle), *Coleus blumei* Benth. var. *verschaffeltii* Lem. (coleus), *Cosmos diversifolius* Otto Ortg. (cosmos), *Dahlia merckii* Lem. (dahlia), *Dactylis glomerata* L. (orchard grass), *Daucus carota* L. var. *sativa* DC. (carrot), *Dianthus barbatus* L. (sweet William), *Glycine hispida* Max. (soy bean), *Lycopersicon esculentum* Mill. (tomato), *Malconia maritima* R. Br. (stock), *Mentha piperita* L. (mint), *Oxalis repens* Thunb. (wood sorrel), *Pastinaca sativa* L. (parsnip), *Pisum sativum* L. (peas), *Phleum pratense* L. (timothy), *Pelargonium domesticum* Bailey (geranium), *Plantago major* L. (plantain), *Pyrus malus* L. (apple), *Rosa* spp. (rose), *Rumex patientia* L. (dock), *Rumex abyssinicus* Jacq. (rhubarb), *Raphanus sativus* L. (radish), *Solanum dulcamara* L. (bittersweet), *Solanum tuberosum* (potato), *Stellaria media* (L.) Cyrill. (chickweed), *Spinacea oleracea* L. (spinach), *Tragopogon porrifolius* L. (salsify), *Tradescantia flu-*

minensis Vill. (tradescantia), *Triticum vulgare* Vill. (wheat), *Tropaeolum majus* L. (nasturtium), and *Zebrina pendula* Schnizl. (Wandering Jew).

LEAF SYMPTOMS OF THE DISEASE

The symptoms of ring spot are restricted to the leaves of most of the host plants, but they may also appear on the stems and fruits in some instances, as will be described later. The symptoms vary considerably on different hosts, but still show a remarkable similarity in practically all cases. Infection on the leaf usually results in the development of rings and lines that spread out in a zigzag manner, and these are the most characteristic symptoms of the disease.

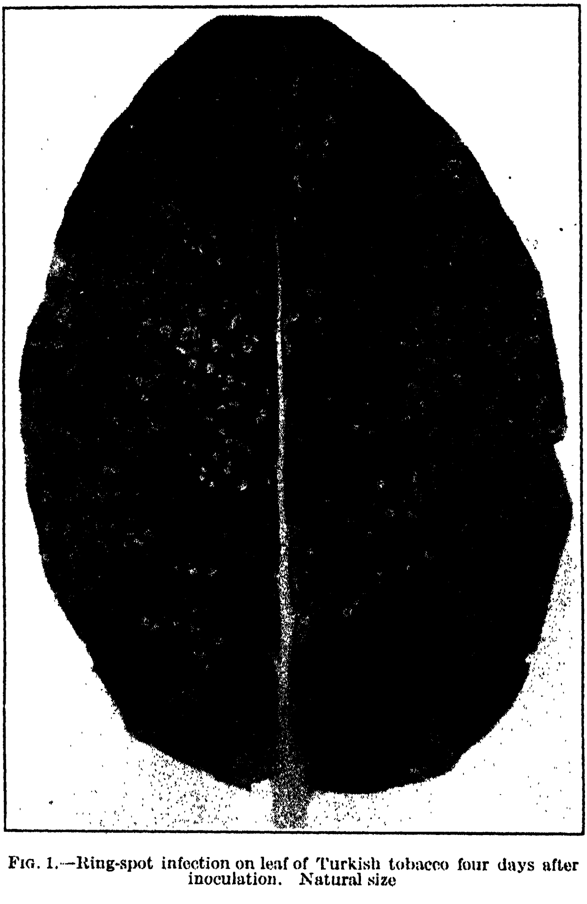

FIG. 1.—Ring-spot infection on leaf of Turkish tobacco four days after inoculation. Natural size

Since the symptoms vary somewhat in even the most closely related plants, it might appear advisable to describe them in detail for each host. This procedure, however, is readily seen to be impractical when the large number of susceptible plants is considered. Therefore, instead of treating each host separately, those which show strikingly similar symptoms will be grouped together with a description of the symptoms that are characteristic of the group.

GROUP 1

The symptoms of the ring-spot disease are quite similar for the following varieties of *Nicotiana tabacum: Atropurpurea, auriculata, brasiliensis, calycina, calyciflora, cavala, colossea, lacerata, latissima, macrophylla, microphylla, purpurea, sanguinea,* and Turkish; and also for the agronomic varieties, Adcock, Little Orinoco, Lizard Tail, Green's Wildfire Resistant, Kentucky Yellow, Macedonian, Maryland, and Warne. The symptoms occur only on the leaves of this group of plants, and are first evident within 48 to 60 hours after inoculation.

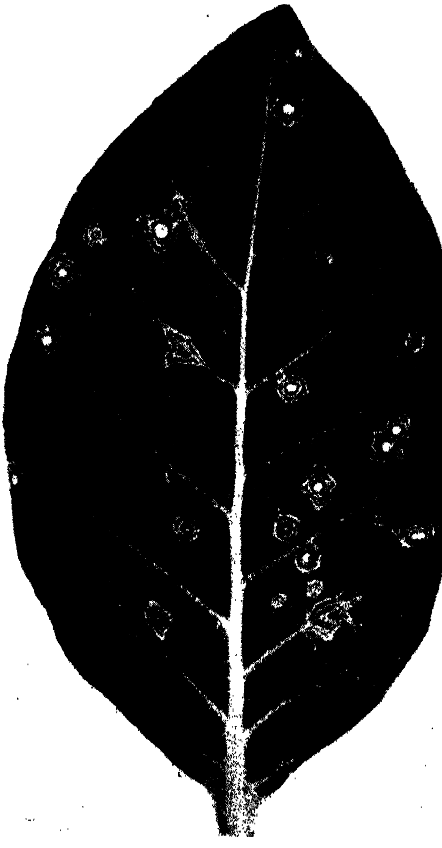

FIG. 2.—Ring-spot infection on Turkish tobacco leaf eight days after inoculation. Note the difference in type of spot produced on the intervein tissue and that along the veins. Natural size

The initial spot consists of a small ring of necrotic tissue surrounding an island of apparently normal tissue. (Fig. 1.) The primary rings are usually not more than 1 mm. in diameter, and in some cases they are so small that they appear as mere dots. The necrotic rings or margins of the young spots appear as translucent lines in transmitted light. They become blanched or brown in color in a few days after they appear. A secondary ring or margin, 3 to 4 mm. in diameter, appears in four days (fig. 2); and a third one, 6 to 7 mm. in diameter, in about five days. This process continues when the spots are thinly distributed over the leaf until finally there are several of these necrotic rings with alternating zones of normal-appearing tissue, as in Figure 3. The center of the spot consists of the primary ring and its island of encircled tissue. The tissue in the center of the spot often dies in five or six days after the spot appears, especially if the primary ring is very small in diameter. In such cases the center of the spot has the appearance of a small dot.

The outlines of the spot vary according to the location. They are circular, as described above, when centered on intervein tissue, but when centered on the larger veins and midrib they are very irregular in outline. (Figs. 2, 4, and 5.) The infection follows the vein and its branches, and the outline of the spot often suggests that of a deeply lobed leaf. (Figs. 4 and 5.)

Infection becomes systemic in 8 to 14 days after inoculation. If some of the leaves of a medium-sized plant are inoculated, local symptoms will appear in 48 to 60 hours, and in about 6 days symptoms will begin to appear on the uninoculated leaves, especially on the upper ones. The infection will continue up the plant, and in 10 to 14 days all the tiny bud leaves will show typical ring-spot symptoms. The symptoms of systemic infection show a marked tendency to follow the midrib and larger veins of the leaves, yet the characteristic circular spots appear on the intervein tissue. (Fig. 5.) As soon as the infection becomes systemic and symptoms appear on all the leaves of the plant—in about 10 to 14 days after inoculation—new leaves will appear on which symptoms may either fail to develop altogether or will develop only slightly, appearing only on the tip half of the leaf. The symptoms gradually fail to develop on the new growth, and those that do develop are located nearer and nearer the tips of the leaves until finally only faint symptoms can be detected at the very tip of the leaf. (Fig. 6.) New leaves produced after this stage

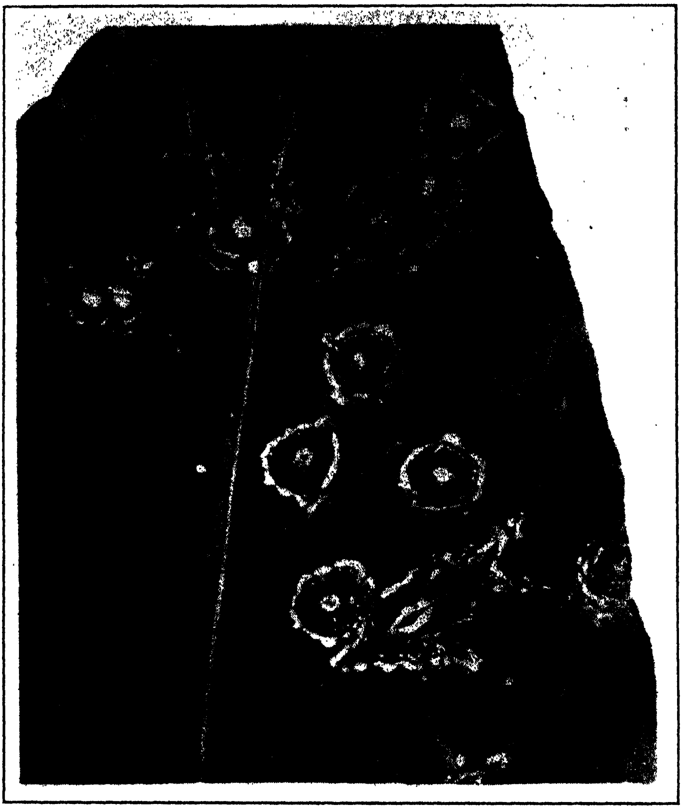

FIG. 3.—Leaf of Green's Wildfire Resistant tobacco showing ring-spot infection three weeks after inoculation. Natural size

has been reached may appear normal, or they may show a faint grayish mottling. In some cases these apparently normal leaves on affected plants develop numerous brown necrotic specks. The margins are necrotic in the first spots that appear on these plants, but as new leaves appear and as the symptoms begin to become less conspicuous the margins of the spots are no longer necrotic but are chlorotic or only of a lighter green color than the normal tissue.

The leaves of plants on which the ring-spot symptoms have become masked appear to be a little thicker and to have more of a leathery texture than those of normal plants. (Fig. 7.) The leaves of suckers that develop from these old plants usually appear normal or show only a grayish mottling. However, typical ring-spot symptoms have appeared on the leaves of such suckers in a few instances. Several attempts have been made to produce ring-spot symptoms on the young leaves of both suckers and plants that have reached the stage of masked symptoms by reinoculating them with virus from severely affected leaves, but the results have been negative in every instance. On the other hand, the juice from these apparently healthy leaves readily produces typical symptoms when used to inoculate healthy plants. This masking of symptoms, or development of immunity, or

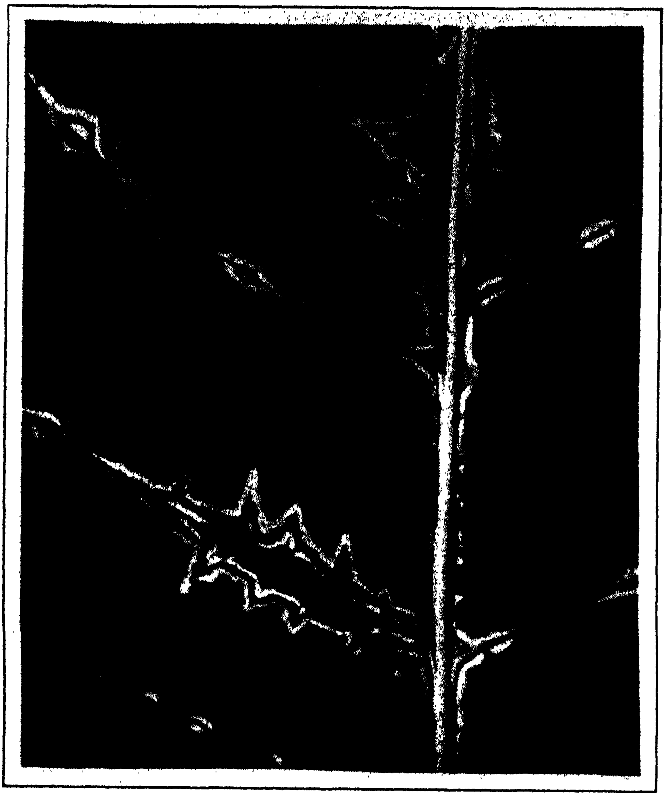

FIG. 4.—Symptoms of ring spot on a leaf of *Nicotiana tabacum* var. *purpurea* as a result of systemic infection. Natural size

whatever it is, seems to hold under greenhouse conditions for practically all the plants tested.

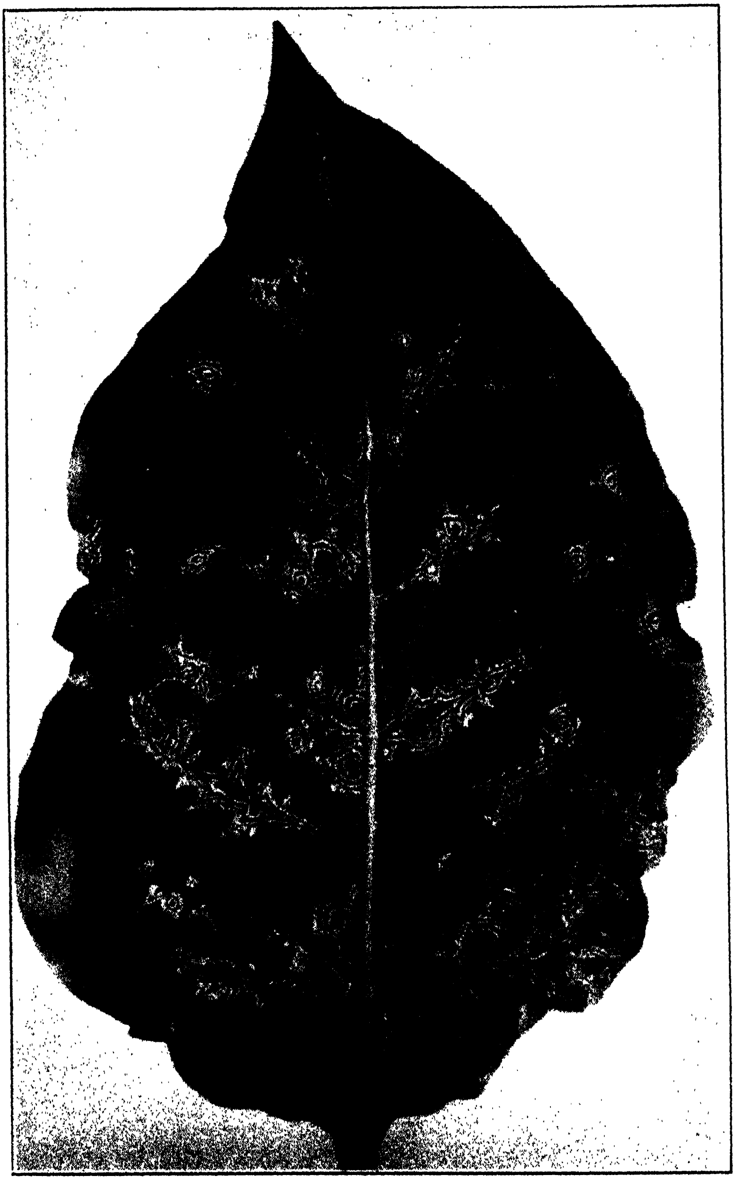

FIG. 5.—Systemic ring-spot infection on a leaf of Turkish tobacco. The lower leaves on the plant from which this leaf came were inoculated five weeks before this leaf was photographed. Natural size

GROUP 2

This group is represented by *Nicotiana rustica* var. English, *N. tabacum* var. *gigantea,* and the Burley strains of *N. tabacum,* namely, Big Burley and Stand-up Burley. The ring-spot symptoms begin to appear on the leaves of these plants in about three days after inoculation. The first sign of infection is the development of a small necrotic spot which is surrounded by a definite light yellow halo. (Fig. 8.) The spots at this stage look almost exactly like wildfire spots, caused by *Bacterium tabacum* Wolf and Foster. The center of the spot may begin as a small brown speck or it may at first be composed of a small ring of necrotic tissue surrounding an island of apparently normal tissue. The tissue within the ring becomes chlorotic and then necrotic, giving the spot its characteristic center. The necrotic centers, 1 to 5 mm. in diameter, are surrounded by a definite light green halo, at the margin of which a broken or a continuous line of necrotic tissue develops. This ring soon becomes surrounded by a light green halo, which in turn develops a necrotic line at its margin. This process continues until several such rings are developed and the spot measures 10 to 20 mm. in diameter. Numerous fine specks often develop in the alternating zones of living tissue.

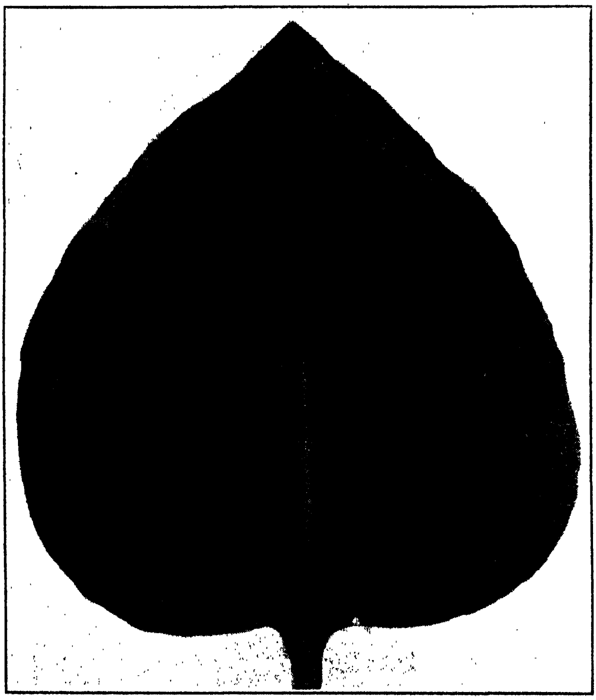

FIG. 6.—One of the top leaves of a Turkish tobacco plant showing the last trace of ring-spot symptoms on its tip. Lower leaves from the same plant were severely affected. Slightly reduced

Infection becomes systemic in 10 to 14 days and the symptoms begin to appear on the bud leaves. The leaves of intermediate size develop circular necrotic spots, or lines of necrotic tissue surrounding circular islands of living tissue, and zigzag lines of necrotic tissue running at random in the intervein tissues. (Fig. 9.) The young bud leaves are often almost completely destroyed. Big necrotic spots appear and the leaf becomes twisted and distorted and never makes much growth. Another characteristic symptom on the leaves of Burley tobacco is the development of irregular lines of necrotic tissue along the midrib and larger veins as shown in Figure 10. There is a marked dwarfing of the entire plant as a result of infection.

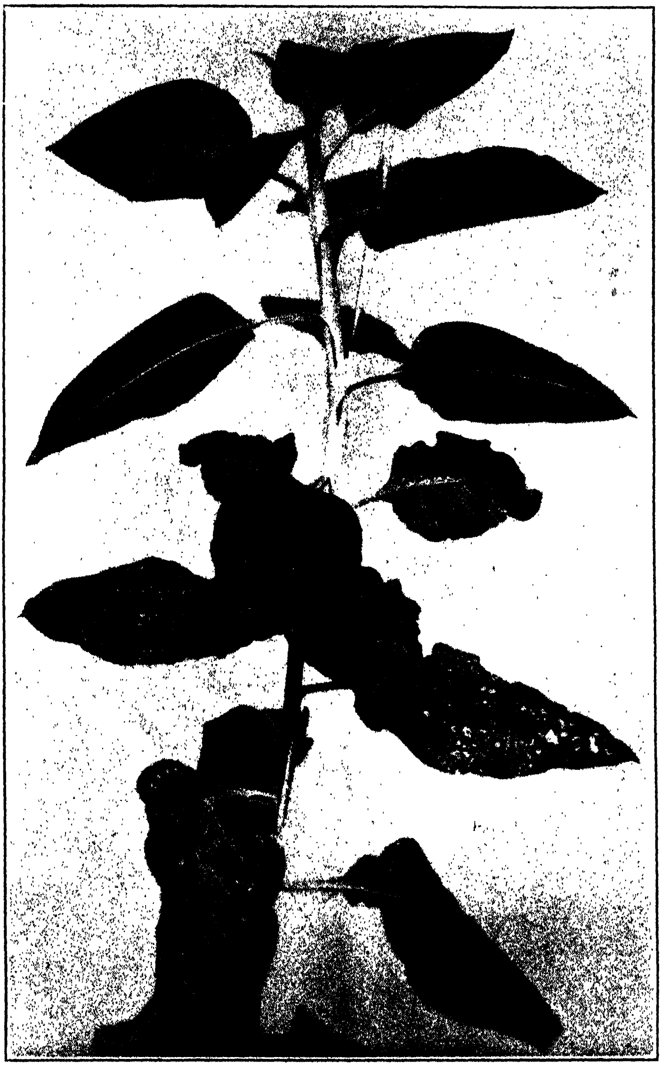

FIG. 7.—Turkish tobacco plant 23 days after inoculation with ring-spot. Note the gradual decline in the development of ring-spot symptoms on the upper leaves until finally the top leaves appear perfectly normal. Much reduced

The plants in this group also, sooner or later, develop an apparent immunity or tolerance to the disease. New leaves develop on which no symptoms appear, and yet the sap from such leaves is quite virulent when used as inoculum on healthy plants.

GROUP 3

This group includes pokeweed, petunia, *Nicotiana langsdorffii*, and *N. rustica* var. Iowa and var. *jamaicensis*. The symptoms of infection on these plants are similar in many respects to those of the plants in Group 1. The spots appear in about three days after inoculation and are composed of an island of normal green tissue surrounded by a continuous line of necrotic tissue which at first has a water-soaked appearance, but after a few days dries out and becomes perfectly white, as shown in Figure 11. The spots on pokeweed usually agree

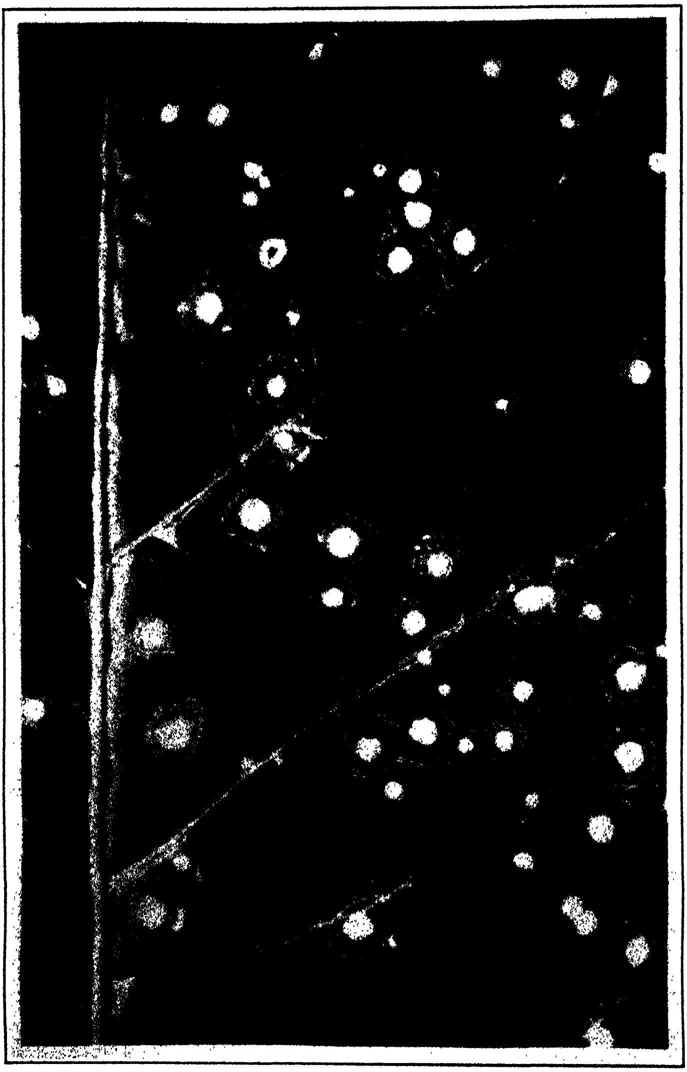

FIG. 8.—Ring-spot infection on leaf of Big Burley tobacco 10 days after inoculation. About natural size

with the above description, but in some cases they are more compact and the marginal lines instead of being composed of continuous lines of necrotic tissue are made up of a series of necrotic specks. (Fig. 12.)

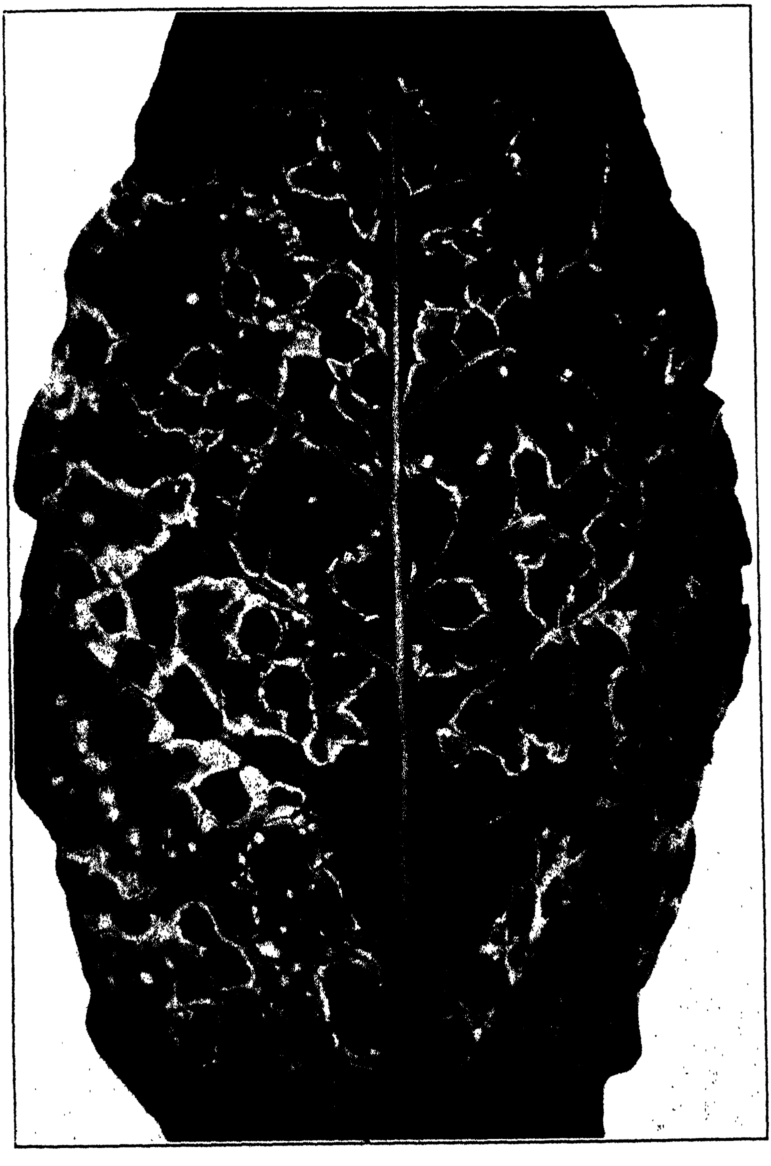

FIG. 9.—Systemic ring-spot infection on one of the top leaves of a Big Burley tobacco plant six weeks after some of the lower leaves had been inoculated. Slightly reduced

The primary rings are followed in two or three days by secondary rings of the same type and the secondary rings are in turn followed by tertiary ones. The tissue intervening between the necrotic margins retains its normal green color. Infection becomes systemic and for a time the new leaves will develop the typical spots, but after two to four weeks new leaves form on which the symptoms develop only faintly with the marginal lines no longer becoming necrotic. This continues until finally no symptoms appear on the new growth. The old spots on pokeweed leaves become masked by a thin velvety growth which is of a Tyrian rose color.

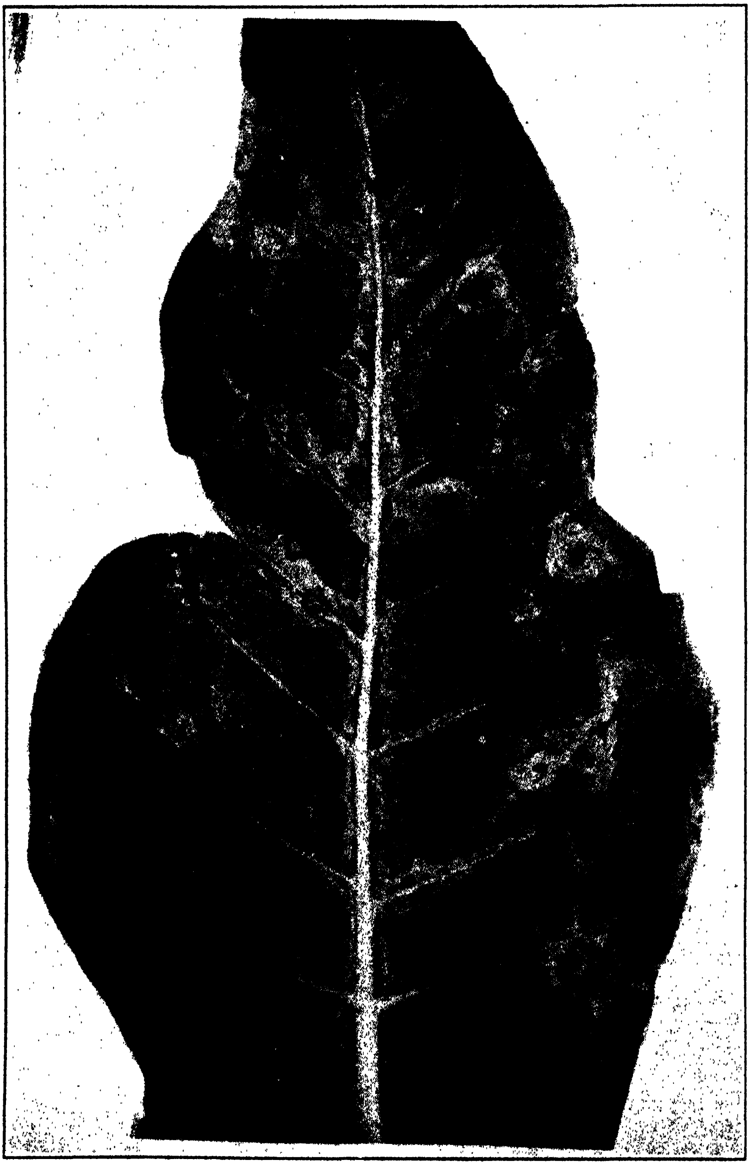

FIG. 10.—Systemic ring-spot infection on leaf of Big Burley tobacco. Note how the necrotic lines follow the midrib and veins of the leaf. Slightly reduced

GROUP 4

This group is represented by a wide variety of plants, all of which seem to be hypersensitive to the ring-spot virus. It includes the following species of Nicotiana: *Clevelandii*, *glutinosa*, *longiflora*, *multivalis*, *plumbaginifolia*, *quadrivalis*, *repanda*, *sanderae*, *suaveolens*, *sylvestris*, and *trigonophylla;* also garden aster, China aster, kidney bean, Lima bean, Broad Windsor bean, castor-oil bean, eggplant, watermelon, Jimson weed, prickly lettuce, horse nettle, New Zealand spinach, ground cherry, and snapdragon.

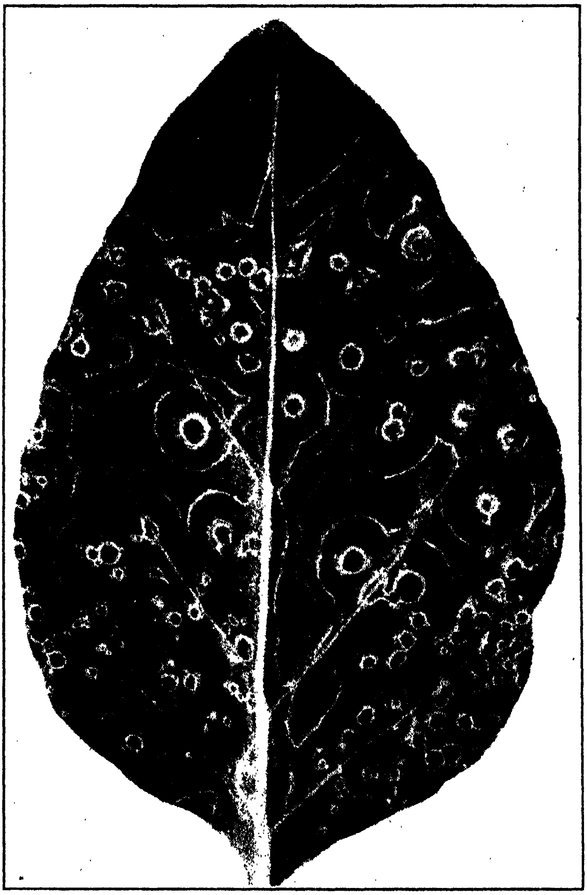

FIG. 11.—Ring-spot infection on leaf of *Nicotiana langsdorffii* three weeks after inoculation. About two-thirds natural size

The most characteristic symptom of infection for this group is the development of spots that are necrotic throughout. The primary infection on certain of these plants, namely, *Nicotiana sylvestris*, *N. repanda*, *N. glutinosa*, Lima bean, garden bean, castor-oil bean, cowpea, watermelon, and horse nettle, appears as definite necrotic spots in three days after inoculation. The spot has a light center about 1 mm. in diameter (figs. 13 and 14) which is surrounded by a band of dark brown tissue. The margin of the spot consists of newly killed tissue which has a black, water-soaked appearance. This marginal tissue turns brown on drying and a new necrotic band is formed. Thus the spots enlarge until they finally coalesce and either kill the entire leaf or large sections of it. (Fig. 15.) The initial symptoms on certain other plants of this group, of which *N. sanderae*, *N. multivalvis*, snapdragon, and New Zealand spinach are typical examples, are typical rings as described for plants in Group 1. (Fig. 16.) The spots remain as alternating zones of living and necrotic tissue for about a week and then the entire spot becomes necrotic and the necrosis spreads until the spots coalesce and kill the entire leaf or large areas thereof. (Fig. 15.) The original necrotic lines can still be seen in the necrotic spots on this leaf.

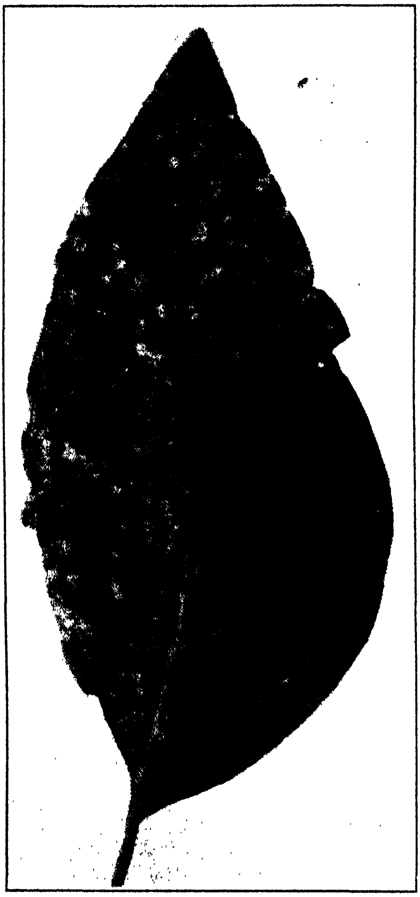
FIG. 12.—Systemic ring-spot infection on pokeweed leaf. Natural size

The foregoing relates to symptoms that develop on the parts of the leaf to which the inoculum was applied. After infection has become systemic in these plants, spots develop on the new leaves that are composed of rings of necrotic tissue intervening between zones of apparently normal tissue. (Fig. 17.) Finally, the symptoms fail to appear on the new growth.

GROUP 5

This group is represented by cantaloupe (Honey Ball and Honey Dew), cucumber (Everbearing and Ideal White Spine), gourd (Nest Egg, Dipper, and Dish Cloth), pumpkin (Cornfield and Cutshaw), and squash (Golden Summer Crookneck and Mammoth White Bush). These plants are very susceptible to ring-spot infection, as shown by the abundant development of symptoms within three days after inoculation. The young spots on the leaves consist of a small yellow to brown pin-pointlike center surrounded by a light-yellow margin or halo. (Fig. 18.) The pin-point center in some cases becomes necrotic, but as a rule the necrosis does not spread throughout the spot. Although the type of spot described above is the most typical for this group of plants, there is still a tendency for definite rings to appear, especially on the leaves to which the inoculum is applied. In some cases the tiny brown centers are surrounded by two or three necrotic rings.

Infection becomes systemic in about 10 days and the halo type of spot appears in great numbers on all the new leaves. (Fig. 18.) In the case of the dipper gourd the leaf is literally covered with light-yellow spots no more than 1 mm. in diameter. These tiny spots have definite pin-pointlike centers that are water soaked in appearance. On squash and Nest Egg gourd the spots are much larger (figs. 18 and 19) but are of the same type. On pumpkin the spots are restricted primarily to the leaf veins. (Fig. 20.) The halo type of spot also appears on cantaloupe, cucumber, and Dish Cloth gourd; but in addition to this definite rings and zigzags often develop. The lines and zigzags are much lighter in color than the normal green leaf tissue but as a rule there is no necrosis.

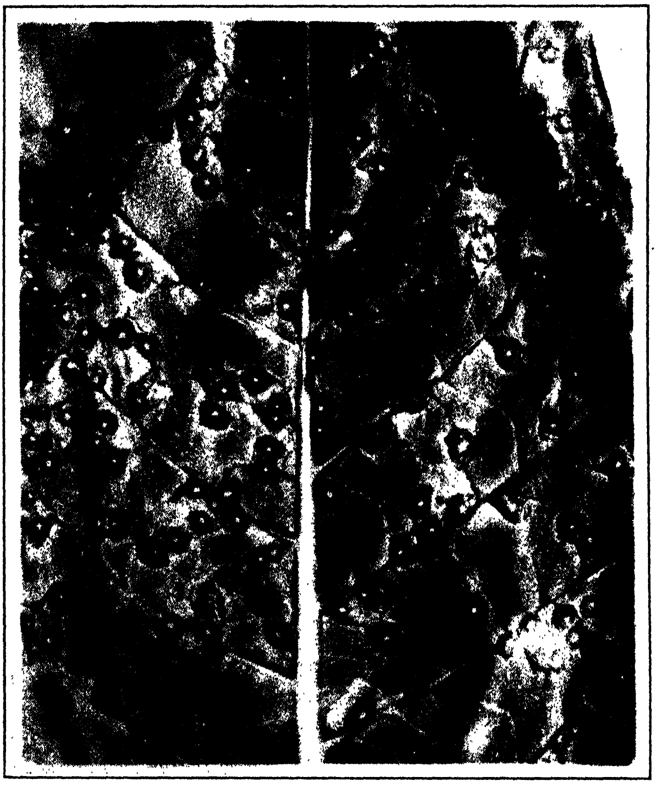

FIG. 13.—Ring-spot infection on leaf of *Nicotiana sylvestris* 10 days after inoculation. Note the light centers and brown margins of the spots. Natural size

GROUP 6

This is a miscellaneous group in which all the remaining host plants have been placed, even though their ring-spot symptoms may differ slightly from each other. In general, infection on these hosts produces either the ringlike spot or the fine zigzag lines that run at random across the leaf. There is not sufficient difference in the symptoms on these plants to warrant separating them into main groups, but they will be placed in subgroups according to their ring-spot symptoms.

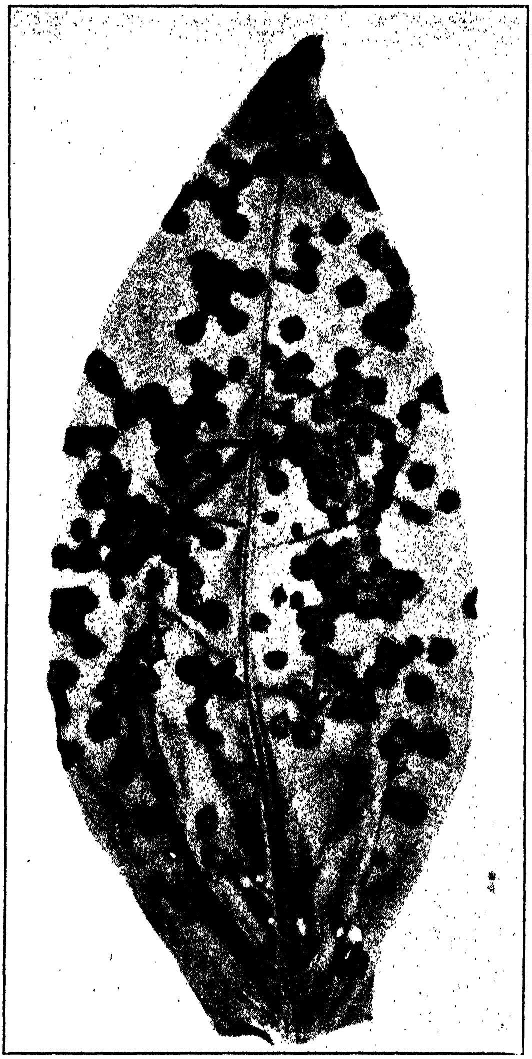

FIG. 14.—Ring-spot infection on leaf of *Nicotiana repanda* two weeks after inoculation. Note the brown necrotic spots. Natural size

Garden beet, sugar beet, Swiss chard, pot marigold, and *Nicotiana tomentosa* develop very faint zigzag lines on their leaves as a result of ring-spot infection. These lines are of a lighter color than the normal tissue but are never necrotic.

Infection on *Nicotiana acuminata*, pigweed, lamb's-quarters, field erigeron, morning-glory, winter cress, small ragweed, giant ragweed, violet, giant pansy, sweet scabious, night shade, apple of Peru, and sweet clover produces definite necrotic rings with the tissue within the ring usually remaining green.

On Spanish needle, African marigold, and smartweed the symptoms develop as very fine zigzag lines which may or may not form inclosures with irregular borders. This type of infection is shown in Figure 21. The tissue in the marginal lines becomes necrotic and turns white.

Infection on Jerusalem cherry results in the formation of both rings and zigzags on the leaves. (Fig. 22, A.) The rings and zigzags are made up of chlorotic tissue which never becomes necrotic. On scarlet sage definite light-green lines develop along the midrib and larger veins of the leaf. This is especially true of the symptoms that develop on this plant as a result of systemic infection. Infection on sunflower leaves produces a large circular spot, composed of a center of normal green tissue surrounded by a broad band of light green. This light green border becomes necrotic with age and takes on a brown color. Some of the spots are a centimeter or more in diameter. Infection becomes systemic and produces brown necrotic

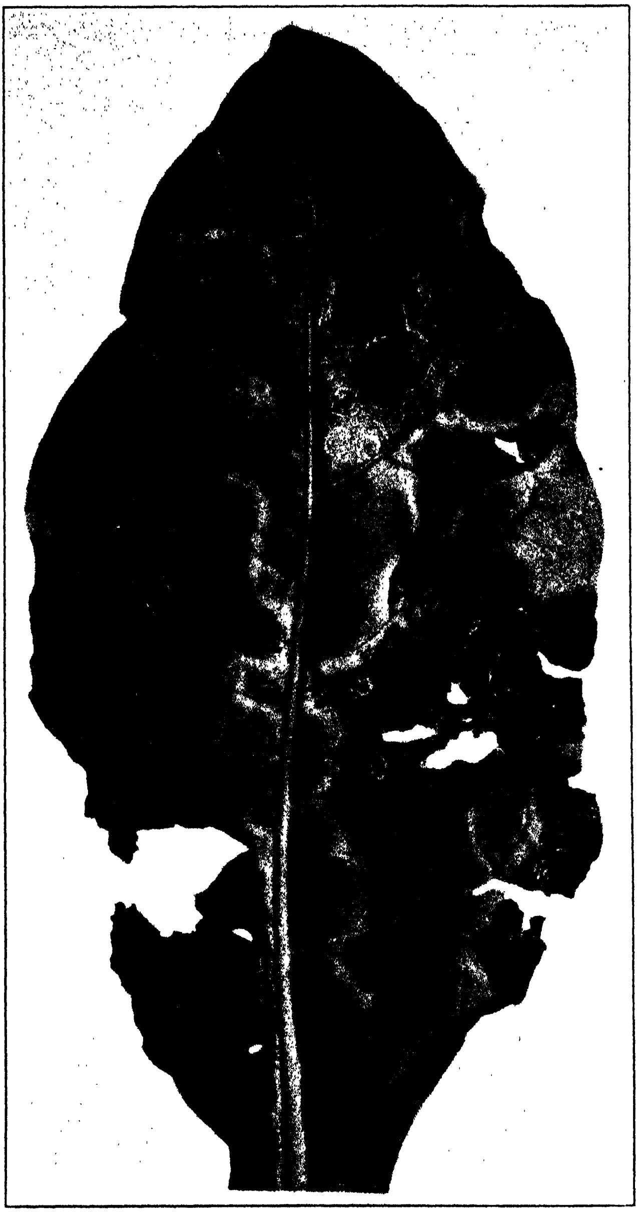

FIG. 15.—Ring-spot infection on leaf of *Nicotiana sanderae*. Note the definite concentric rings which are later followed by a complete necrosis of all the surrounding tissues. Four-fifths natural size

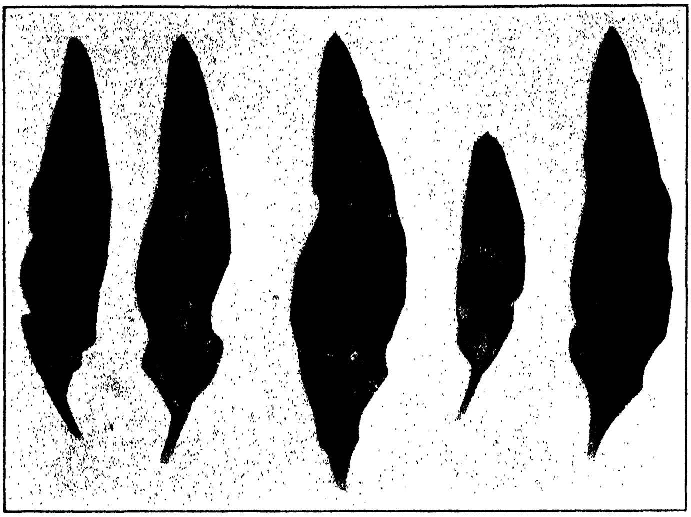

FIG. 16.—Ring-spot infection on snapdragon leaves a week after inoculation. Natural size

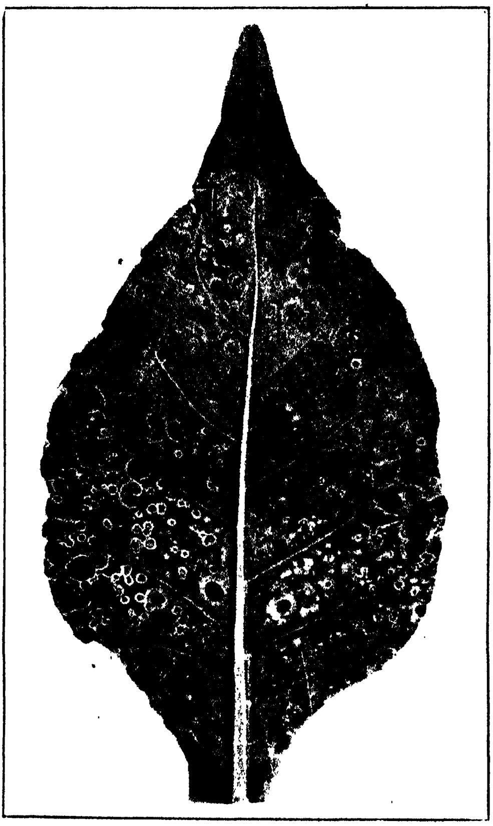

FIG. 17.—Systemic ring-spot infection on leaf of *Nicotiana plumbaginifolia*. Natural size

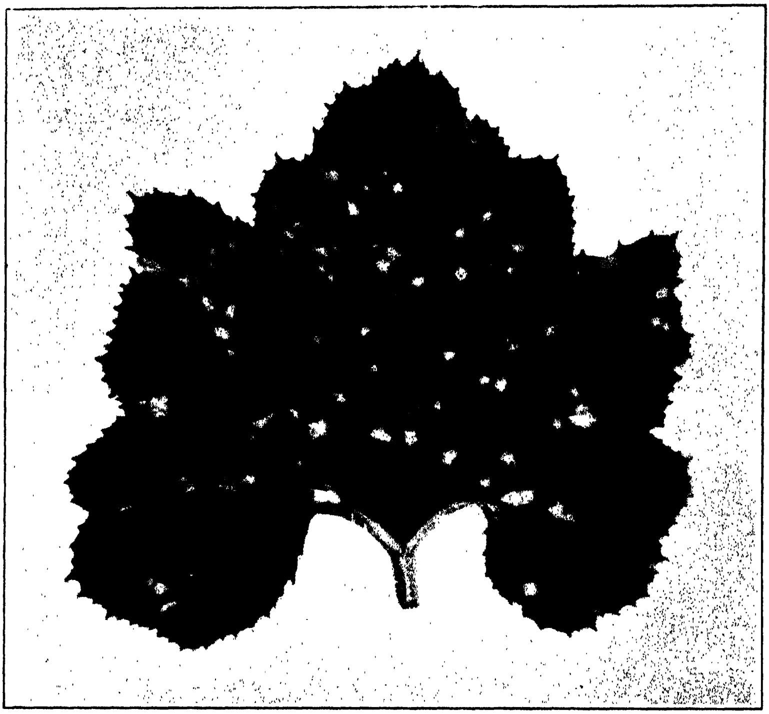

FIG. 18.—Systemic ring-spot infection on leaf of Golden Summer Crookneck squash. Natural size

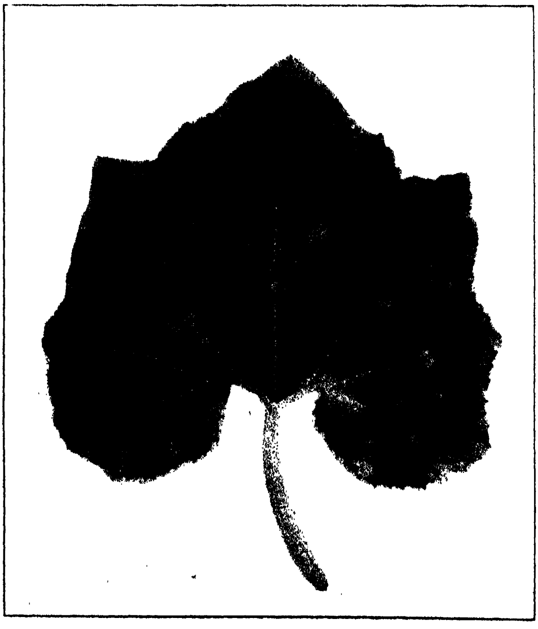

FIG. 19.—Systemic ring-spot infection on leaf of Nest Egg gourd. Natural size

rings on the involucral bracts of the flower. The symptoms on okra leaves are similar to those on sunflower except that the margins of the spots never become necrotic. No definite ring symptoms develop on the leaves of zinnia and head lettuce, and yet infection becomes systemic in these plants. The new leaves that develop after these plants have been inoculated show a marked stunting and in some cases are slightly crinkled but no other symptoms have been observed. The juice from such leaves, however, produces typical ring-spot symptoms when used as inoculum on Turkish tobacco.

STEM SYMPTOMS OF THE DISEASE

As a rule, symptoms do not occur on the stem of the host plant as a result of ring-spot infection; but in exceptional cases the stem shows marked symptoms and the entire plant may be killed. Stem lesions are most likely to occur on plants that are hypersensitive to the ring-spot virus, such as those listed in Group 4.

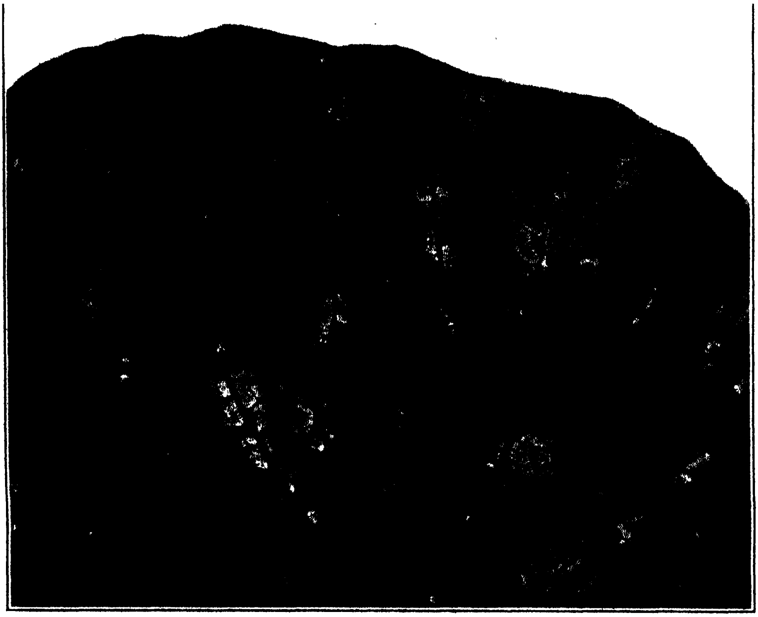

FIG. 20.—Systemic ring-spot infection on leaf of cornfield pumpkin. Natural size

Representatives of the following plants have been killed outright in one or more of the inoculation experiments: *Nicotiana clevelandii*, *N. glutinosa*, *N. longiflora*, *N. multivalvis*, *N. plumbaginifolia*, *N. quadrivalvis*, *N. sanderae*, and *N. sylvestris*. The symptoms first appear on the leaves as dark circular necrotic spots. Then dark sunken lesions develop on the leaf petioles, and in about 10 days similar lesions appear on the main stem of the plant, in some cases extending its full length. The plant becomes twisted and dies in two or three weeks. The vascular tissues of the stem and roots become black before the plant dies. One of the *N. glutinosa* plants was killed only to the ground line and later sent up suckers. This is very unusual, however, because the roots are usually destroyed in cases of such severe infection.

As noted, the above-named plants may or may not be killed outright by the ring-spot infection; but the following species are apparently always killed outright: Snapdragon, kidney bean, Lima bean, Broad Windsor bean, cowpea, and New Zealand spinach. The necrotic spots that appear on the leaves of these plants are followed in two or three days by an infection of the leaf veins and petioles. The veins turn black and shrivel, and the lesions on the petioles become dark and sunken; and in the case of kidney bean, the infection looks very much like that of anthracnose. Dark sunken lesions also appear on the main stem, and the plants die in 10 to 14 days.

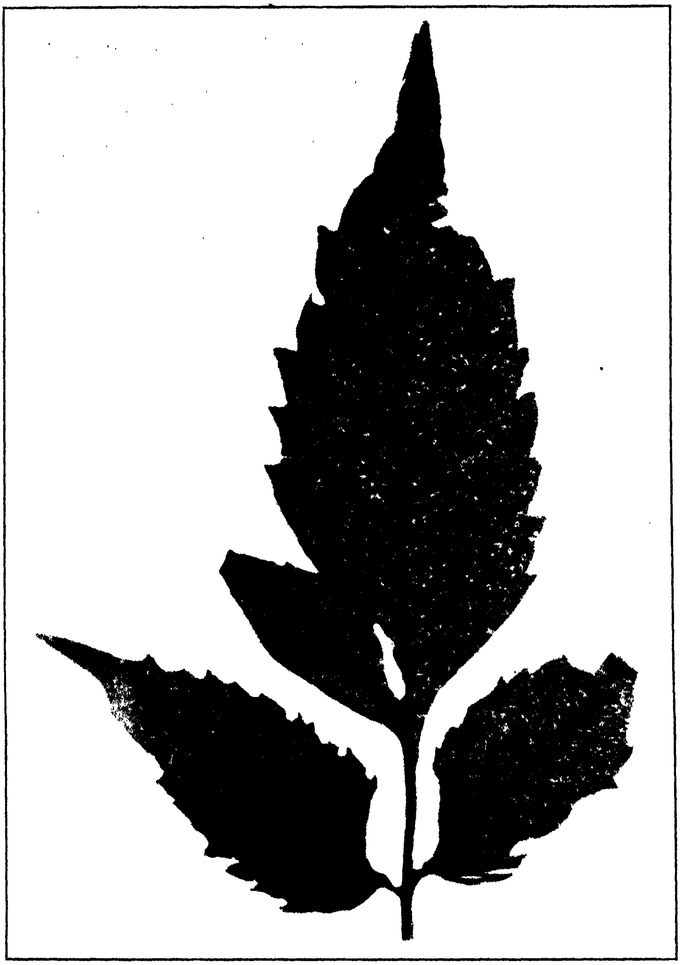

FIG. 21.—Ring-spot infection on leaf of Spanish needle. Note the very fine lines on this leaf. About natural size

FRUIT SYMPTOMS OF THE DISEASE

Symptoms of ring-spot infection have recently been obtained on the fruits of some of the hosts; and, so far as the writer knows, this is the first time the symptoms have ever been observed on fruits. These observations were made on the fruits of Nest Egg gourd and Golden Summer Crookneck squash, which were grown in the green-

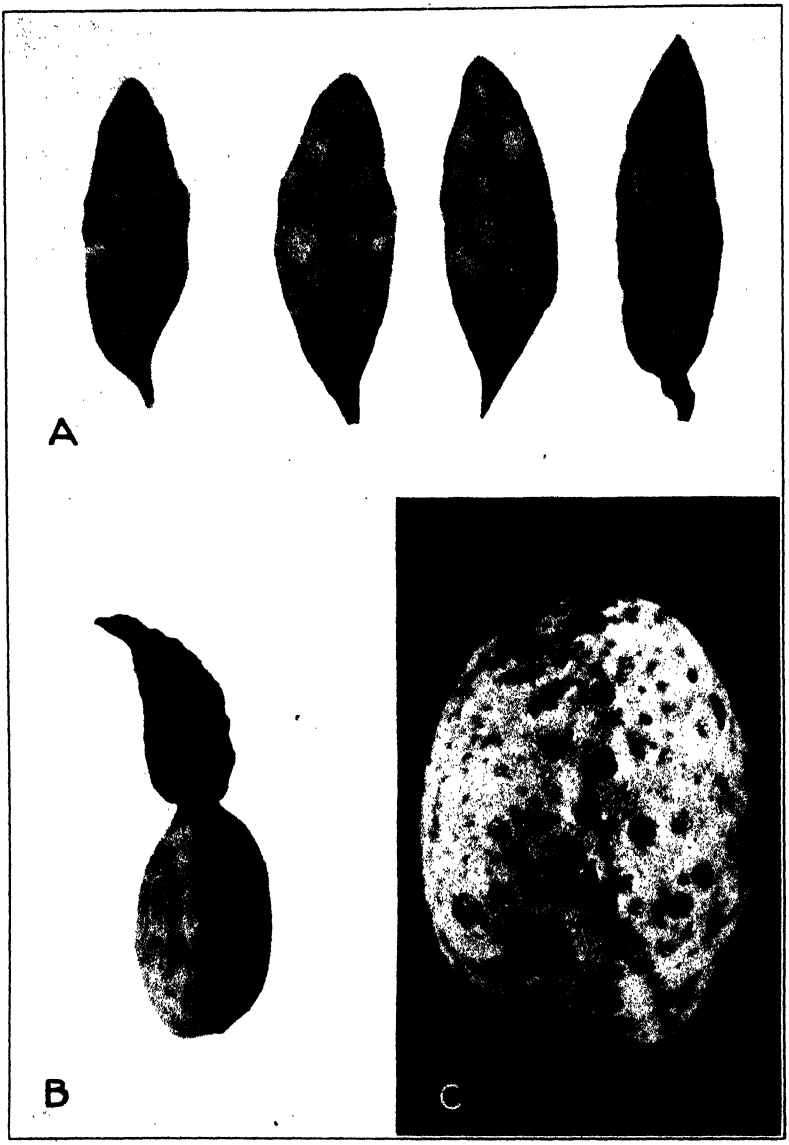

Fig. 22.—A, systemic ring-spot infection on the leaves of Jerusalem cherry. B, ring-spot infection on a young Nest Egg gourd; infection came from the vine. C, mature fruit of Nest Egg gourd showing ring-spot symptoms as a result of systemic infection in the plant on which it was produced; note the concentric rings of the spots. All natural size.

house. The leaves of these plants were inoculated with the ring-spot virus when the plants were about 2 weeks old. Infection became systemic, and all the new leaves developed typical symptoms; and when the young fruits appeared they also showed the characteristic ring-spot symptoms.

The fruits show the ring-spot symptoms very early and often drop when they are only 1 or 2 inches in length. The symptoms first appear as small, circular, water-soaked spots not more than 1 mm. in diameter. (Fig. 22, B.) They become depressed in a few days and give the surface of the fruit a pitted appearance. (Fig. 23.) These pits become encircled by a very narrow line of water-soaked tissue in four or five days. These circular margins or rings are more easily seen when thin horizontal sections are cut from the surface of the fruit. The spots penetrate to a depth of 2 to 3 mm. as a rule, and in some cases can be traced all the way to the seed cavity. The spots develop a deep green pigment in contrast to the white or yellow pigment of the normal tissue. (Fig. 22, C.) Three of the infected Nest Egg gourd fruits grew to maturity, and the spots which had at first been depressed gradually became elevated and finally appeared as pimples on the surface of the mature fruit. (Fig. 22, C.) The spot in its final stage is composed of a small elevated center surrounded by one or more definite rings.

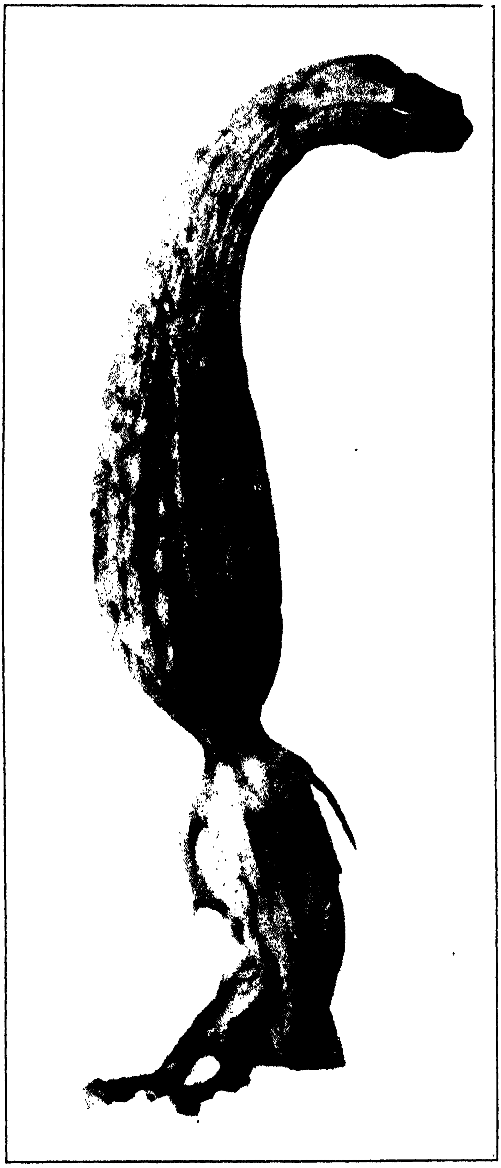

FIG. 23.—Ring-spot infection on a young Golden Summer Crookneck squash. Infection was systemic in the plant on which this fruit was produced. Natural size

DISCUSSION OF RESULTS

The results of the inoculation experiments show that the ring-spot virus has a very extensive host range. They also show that the virus is very specific in its infective properties, since so many of the plants that were tested failed to develop infection, and especially the solanaceous plants, pepper, potato, and tomato. One should naturally expect plants that are so closely related to tobacco to be susceptible. Repeated attempts were made to infect these plants but the results were always negative.

The list of susceptible plants includes horse nettle, pokeweed, and sweet clover, all of which may play an important part in the overwintering of the ring-spot virus. Horse nettle is a common weed in tobacco fields throughout Virginia, and it may serve as a constant source of infection.

Pokeweed is common in old plant-bed sites and around the borders of tobacco fields. This plant is very susceptible to ring-spot infection, and the sap from its infected leaves is very infectious on tobacco. Infection becomes systemic in this plant, but the symptoms fail to appear on the new growth a short time after the plant becomes infected. It appears possible, therefore, that apparently normal pokeweed plants may carry the virus and act as a reservoir of inoculum for tobacco.

These results also help to explain why ring-spot is usually present on tobacco that is grown in old garden and building sites. Pokeweed, Jimson weed, and horse nettle are always in evidence around such places and, if infected, may serve as a source of infection for tobacco.

Ring-spot infection is often very severe on tobacco crops that follow clovers and alfalfa in rotation. Such fields have been observed in which 50 per cent of the plants were heavily affected with ring-spot. These observations suggested that the leguminous crops were in some way responsible for the infection on tobacco, especially when sweet clover was found to be very susceptible to infection by the ring-spot virus. Natural infection has recently been found rather commonly on sweet clover that is growing on the experiment station plots, and in rotation on the college farm. Inoculum from both the artificially inoculated and the naturally infected sweet-clover plants readily produced the typical ring-spot symptoms on tobacco. Other clovers are no doubt susceptible to infection. These results help to account for infection on tobacco following clover in rotation.

The virus loses its potency at ordinary temperatures as soon as the infected plant tissue decays or becomes thoroughly dry, and in the expressed sap in about 24 hours. It seems, therefore, that the virus must overwinter in biennial or perennial plants, or in the embryo of infected seed. Since the virus appears to retain its potency indefinitely regardless of temperature as long as it is contained in living tissue, it seems that the embryo of the seed should carry it through the winter months. The systemic nature of infection should provide ample opportunity for the seed to become infected. The lesions on the young squash and gourd fruits penetrate the wall of the ovary and may extend to the placental tissue. Embryonic seeds might possibly become infected by their proximity to infected tissue.

Plants seem to develop an immunity to the ring-spot disease. Symptoms appear in abundance when the plants are inoculated and infection soon becomes systemic, as shown by the development of symptoms on the leaves that were uninoculated. New growth takes place and the ring-spot symptoms will appear on it. This process continues for 10 days to 2 weeks and then symptoms appear only faintly on the new leaves and suckers. Finally, the symptoms fail to appear on the new growth and the leaves appear normal. Attempts have been made to produce symptoms on these leaves by reinoculation with virus from heavily infected leaves from other plants, but without success. The sap from these apparently normal leaves is just as virulent as that from leaves that show the disease symptoms, indicating that the virus is present in such leaves even though the disease symptoms fail to appear.

SUMMARY

The ring-spot virus is capable of producing disease symptoms, more or less typical, on a wide variety of plants.

Plants of 72 genera have been inoculated, 38 of which developed infection, representing 17 families, namely, Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Dipsaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Phytolaccaceae, Polygonaceae, Scrophulariaceae, Solanaceae, and Violaceae.

The first symptoms appear in about 3 days after inoculation and infection becomes systemic about 10 days later.

The symptoms of the disease are restricted to the leaves of the majority of the plants, but they may also appear on the stems and fruits of some of the hosts.

Symptoms appear on the fruits of Nest Egg gourd and Golden Summer Crookneck squash as a result of systemic infection of the plant.

Certain plants are often killed outright as a result of ring-spot infection, namely, *Nicotiana clevelandii*, *N. glutinosa*, *N. longiflora*, *N. multivalvis*, *N. plumbaginifolia*, *N. quadrivalvis*, *N. sanderae*, *N. sylvestris*, Broad Windsor bean, Black-eye cowpea, kidney bean, Lima bean, snapdragon, and New Zealand spinach.

Infection has been found occurring naturally on sweet clover and the commercial varieties of tobacco.

Tobacco (*Nicotiana tabacum*) has been infected with virus recovered from the following plants: *N. clevelandii*, *N. glutinosa*, *N. langsdorffii*, *N. plumbaginifolia*, *N. repanda*, *N. sanderae*, *N. sylvestris*, Jimson weed, garden petunia, horse nettle, cantaloupe, cucumber, squash, pumpkin, gourd, calendula, head lettuce, garden zinnia, kidney bean, garden beet, winter cress, pokeweed, snapdragon, and sweet clover.

BACTERIAL POCKET DISEASE OF THE SUGAR BEET[1]

By NELLIE A. BROWN

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INTRODUCTION

While the intensive work with crown gall on various hosts was in progress some years ago, galls on sugar beets received attention and requests were sent to beet growers for sugar beets showing any sort of excrescence. In 1910 sugar beets were received from Holly, Colo., having at the crown definite galls, which were thought to be crown galls. Many of these did not show the typical features of crown gall, most of the outgrowths being deeply indented nodules, but because they were definite tumors at the crown no question as to their nature was raised at the time. Some of the galls were more or less globose and looked much like crown gall, but many were made up of numbers of small nodules, which grew around the crown and looked like cultivation wounds.

When the galls were cut across, a condition unlike crown gall was noted. There were brown areas inside instead of sound white tissue, and surface lesions from which the discoloration could be traced to the interior. Some of the tumors were fairly smooth on the outside and had no visible breaks in the surface, but when they were cut across, large brown areas, which could be traced for some distance in the interior, were seen. In every cut gall the discoloration could be traced from the outer part to the inner, where it became lost in sound white tissue.

Frequently the edges of the white tissue adjacent to the brown watery places were discolored red or purple. When a cut was made through the brown tissue a mucilaginous substance often oozed out. All the galls received had the brown interiors. Upon cutting the tissue back from the surface where the discoloration was darkest and of greatest extent to the point of least infection in the interior, it was found that the spots were lighter colored and had a water-soaked appearance. These areas were usually circular. There were rather large cavities in the badly diseased places, smaller ones in those less diseased, and very tiny ones in the lighter colored areas, which were most recently infected. Some of the light-colored pieces of tissue with cavities could be broken or cut out intact.

ISOLATIONS AND INOCULATIONS

Razor sections of the brown areas with pocketlike spaces were examined under the microscope, and motile bacteria in great numbers were found. (Pl. 1, C and D.) In the belief that this browning was a secondary infection, which had occurred in true crown-gall

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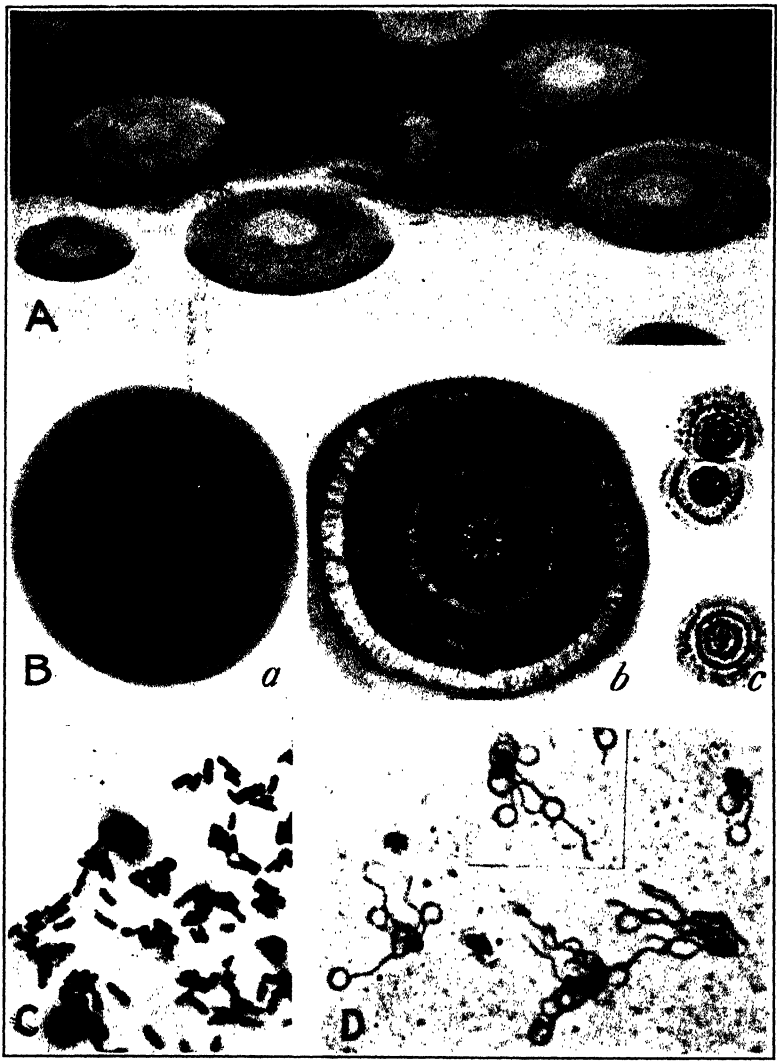
[1] Received for publication June 12, 1928; issued October, 1928. "Bacterial pocket disease" has been substituted for the term "tuberculosis" originally used because the brown pockets in the white tissue are considered the most characteristic feature of the disease.

tissue, the white and light-colored soundest tissue available was used for isolating in the hope of obtaining the crown-gall organism. Repeated platings were made from the parts of the gall which showed no discoloration. No crown-gall colony appeared, but there were numerous colonies of another type. Becoming suspicious of this constant type of colony, the writer poured plates from the parts slightly discolored and also from those of a dark color. The same type of colony (pl. 1, A) appeared throughout the different platings.

Colonies were observed in 21 to 28 hours after the plates were poured. They were very numerous on plates poured from the tube containing the macerated tissue but scattering on the dilution plates, on which they could be studied well. They were buff colored, 2 to 3 mm. in diameter, shining, mostly smooth, some irregular, but most of them round, thin with a thickened place in the center, and with rather inconspicuous concentric rings. Three days after the plates were poured, the colonies were yellow, 4 to 6 mm. in diameter, some smooth on the surface, and others rough except at the outer rim. The concentric rings were not very distinct and usually there were not more than two of them. In November, 1910, 30 young sugar beets growing in the greenhouse were inoculated with cultures from these isolations, and in a month there were ¾-inch galls on 50 per cent of them. The outgrowths were rather flat, rarely hemispherical, but frequently knobbed like the galls resulting from inoculations with the peach, hop, and daisy strains of *Bacterium tumefaciens*.

More specimens of this type of gall were received in November, 1910. The second lot came from Garden City, Kans. (fig. 1), and the third from Rocky Ford, Colo. The galls occurred on the top and sides of the crown and at various intervals on the beet down to the tip. As the tumors were fewer and smaller toward the tip, it was concluded that the infection had not begun at the root tip and worked up, but that it had worked down from the crown. Galls from both lots of beets were used for isolating, and the same buff-colored colonies, changing later to yellow, grew on the plates of both sets. There was no crown-gall colony present. Inoculations with subcultures from these two sets of plates were made on growing sugar beets (24 from the Garden City and 22 from the Rocky Ford lot). Typical tumors with the browned interiors resulted in 10 of the 24 Garden City and in 6 of the 22 Rocky Ford inoculations. The percentage of infection was never so large with this organism as with the crown-gall organism, the latter being usually almost 100 per cent in many strains. With this new gall or tubercle-forming organism, the percentages were in these two cases 41 and 27, respectively, which are high enough, however, to indicate positive results. Later it was found that when the soil was rich in nitrogenous fertilizers the percentage of galls was considerably higher.

The organism was reisolated from the Rocky Ford galls produced in the greenhouse. The yellow colonies appearing on the plates were of two types, some with smooth and others with wrinkled surfaces. In the previous isolations some of the colonies had roughened surfaces but none were distinctly wrinkled like these. The pathogenicity of the wrinkled colonies was tested by inoculating 10 sugar beets with subcultures and that of the smooth colonies by inoculating 16 sugar beets with subcultures. The wrinkled type proved as



A.—Agar plate colonies, smooth type, photographed with oblique light. × 10.
B.—Different varieties of smooth-type colonies: *a*, common growth; *b*, ringed; *c*, shadow occurring at bottom of plates; the colonies are like *b* when the agar is cut and they are able to grow to the surface. × 10.
C.—Bacteria stained in diseased tissue. × about 1,500.
D.—Flagella stained by Casares-Gil's method. × about 2,500.

infectious as the smooth one. Eight of the beets inoculated with the wrinkled type of colony and 14 of those inoculated with the smooth type became infected. These inoculations with reisolations were made in September, and the percentages of infection were high, due to good growing conditions and a better knowledge of soil requirements of the organism.

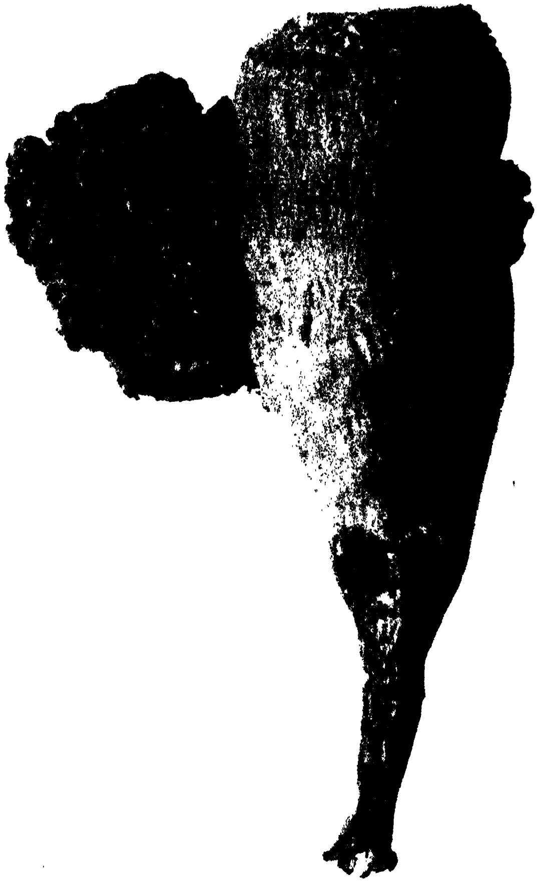


FIG. 1.—Bacterial pocket disease of sugar beet from Garden City, Kans. Photographed Nov. 7, 1910. Natural size

Later both wrinkled and smooth colonies grew on plates poured from a bouillon culture of a wrinkled colony. The size of the colonies varied according to the number on the plates. Those on very thinly sown plates were often 5 to 7 mm. in diameter in three to four days. Besides these round colonies with smooth and wrinkled surfaces there was another type, yellow in color but irregular in outline. Slight marginal irregularities in colonies had been noted before but they were not so pronounced. This colony had a round center of a deep-buff color with a papillate surface, from which rootlike branches extended. Inoculations with the lobed colony proved that it was infectious also, as four of the five sugar beets inoculated with it produced the typical lesions. Plates were made from a bouillon culture of the irregular colony and on them all three types of colonies appeared, most of them being the irregular rhizoid type.

In August, 1912, sugar beets were received from Garden City, Kans., one of which had a ½-inch gall on the crown, with a smaller one adjoining it. On this beet there were six other small nodules that resembled galls, and the condition was considered a case of crown gall. Microscopic examination, however, disclosed that this was not crown gall, but the type first found in 1910. Plates were poured, and in 24 hours colonies were up on them. These were of two types, a lobed colony and a round one, but instead of being buff colored they were white, appearing bluish in transmitted light. This white color was rather disturbing, but the next day it became yellowish and three days later a decided yellow. The colonies had barely perceptible concentric rings. They were thinner than the colonies from the Colorado beets and seemed to grow more rapidly. The lobed type was not smooth on the surface like the round one.

In September, 1912, more beets with galls came from another source at Garden City, Kans. (Fig. 2.) Colonies on the plates poured from these galls were typically buff colored when first up and yellow in 24 hours. The three types—round smooth, round wrinkled, and lobed—appeared on this set of plates. Inoculations with colonies from the two Garden City isolations produced the typical galls or tubercles.

A note was published about this disease in the first bulletin dealing with the crown-gall organism.[2] It was pictured, its cultural characters were stated briefly, and it was named *Bacterium beticolum*.

Few outbreaks have been reported since 1912, and it was supposed that the disease had practically died out until November, 1923, when it appeared to a marked extent in the sugar beets grown at the Arlington Experiment Farm, Rosslyn, Va. (Fig. 3.) By a close observer or one familiar with its appearance, the disease can be noted while the beets are growing in the fields. It is usually, however, not until harvest when the beets are handled and sorted that the infection is found. As stated before, many of the tumors are of a shape and size to be mistaken for cultivation-wound calluses and often pass as such. Wound calluses, however, which consist of sound white tissue, do not carry with them the menace to sugar making that these galls do.

There were 10 full-grown sugar beets from the Arlington farm affected with the tubercular outgrowths. Some of these could be seen before the beets were pulled, as the crowns protruded a little from the ground. The soil in which these beets grew received the following treatment before the seeds were planted: A crop of winter rye

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[2] SMITH, E. F., BROWN, N. A., and TOWNSEND, C. O. CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Dept. Agr., Bur. Plant Indus. Bul. 213: 194. 1911.

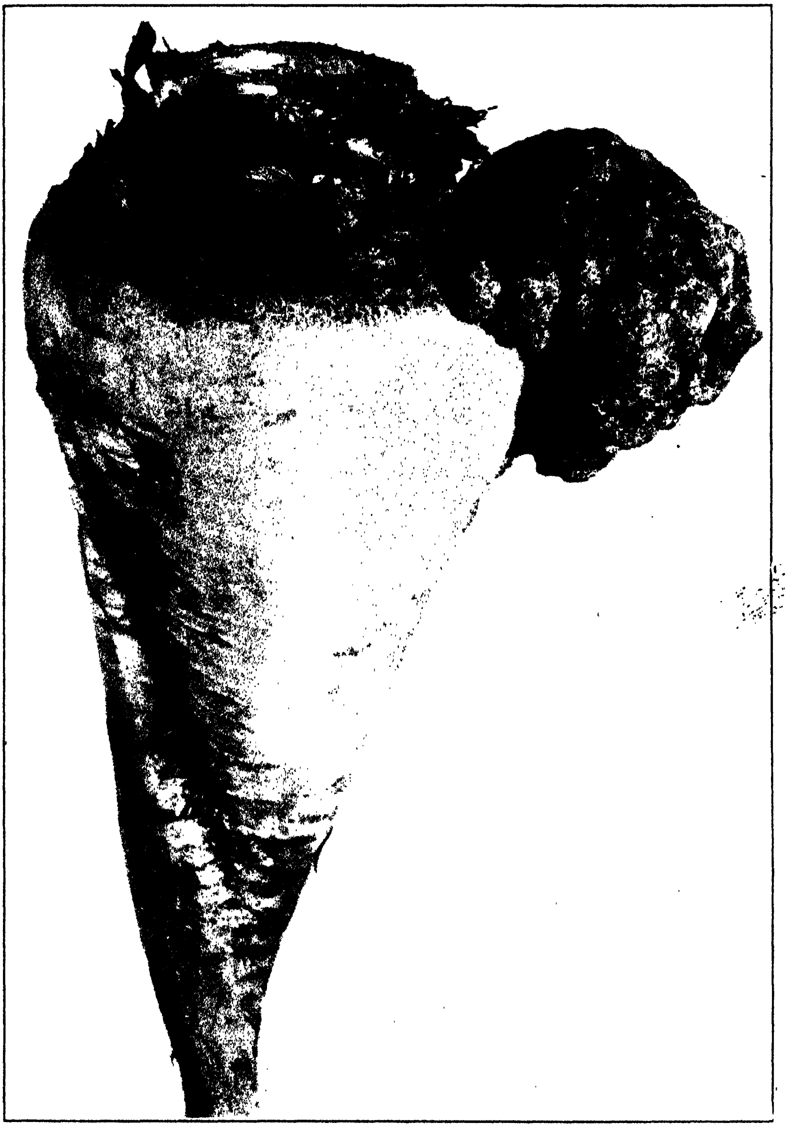

FIG. 2.—Bacterial pocket disease of sugar beet from Garden City, Kans. This type of infection can easily be mistaken for crown gall. Photographed Oct. 2, 1912. About three-fourths natural size

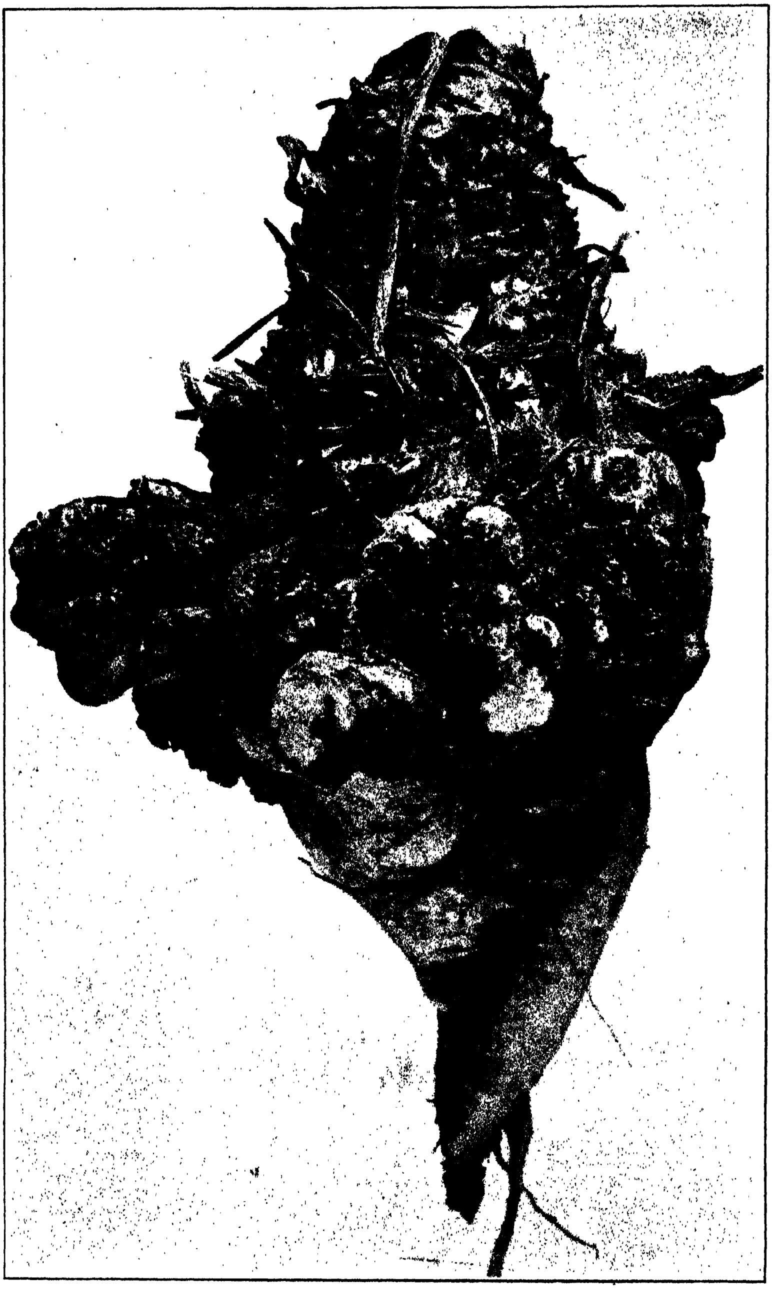

FIG. 3.—Bacterial pocket disease of sugar beet from Arlington Experiment Farm, Rosslyn, Va. Photographed Nov. 3, 1923. One-half natural size

was plowed under, and after a month had elapsed, manure was also plowed under. No other fertilizer was used, but a few weeks before planting a coating of lime was spread over the soil. Plates were made from 7 of the 10 beets, and the same yellow organism was recovered from each beet. Some of the plates contained pure cultures. The colonies were buff colored when they first appeared, as in the isolations of 1910 to 1912, and changed to yellow when older. Some had concentric rings (pl. 1, A, and B, *b*), but most of them were without rings (pl. 1, B, *a*); some had a thickened or papillate center; some were slightly irregular, but most of them were round and had a bluish margin in transmitted light. The shadow colonies were bluish (pl. 1, B, *c*) and the surface ones yellow. There were a few colonies with fish-scale markings as in the first platings of 1910. Forty-two sugar beets were inoculated with isolations from these different beets, and in one to two months 32 of them became infected with galls varying in size from 0.5 to 2 inches in diameter. When cut across all had the brown pockets (fig. 4, A) and viscid exudate. There seemed to be no difference in the infective nature of the different types of colonies, the ringed and those without rings inducing the tubercles with like facility. Reisolation plates were made from these various galls at different times, and 36 sugar beets were inoculated with the reisolation colonies. Twenty-three typical outgrowths resulted (fig. 4, B), one of which was 5 by 4 by 2 inches. The reisolation colonies were of the typical yellow color, but in one set there appeared among the yellow ones a hyaline to white colony, which in transferring was found to be rubbery. When older it became cream colored, but not yellow. Nine sugar beets were inoculated with two of these white rubbery reisolation colonies, and in two weeks four fair-sized galls with pockets were produced. (Fig. 4, C.) One of the infected beets was replanted, and two months later the outgrowth was nearly 4 inches in diameter.

Young tomato plants, Ricinus, nasturtiums, calendulas, Paris daisies, Pelargoniums, garden beets, and Bryophyllums were also inoculated with infectious cultures from the various isolations. No outgrowths occurred on any of these plants except the garden beet. The crown-gall organism, *Bacterium tumefaciens*, however, is infectious to all these hosts.

DISTINCTION BETWEEN BACTERIAL POCKET DISEASE AND CROWN GALL OF SUGAR BEETS

The common type of bacterial pocket disease as observed by the writer is one in which the outgrowths occur at the crown in nodules either singly, in groups, or coalesced. They are not always at the crown, but that is the usual position. The individual nodules are 1 to 3 cm. across and about 1 cm. thick. Frequently, however, the disease has the outward appearance of crown gall on sugar beets. (Fig. 2.) The tumor, instead of spreading over a large part of the crown in the form of nodules, occurs at one point on it, is much larger, and assumes the globose form characteristic of crown gall. In this form it can not be distinguished from crown gall except by cutting across and examining the tissue. Crown-gall tissue is white and sound. Bacterial pocket tissue is brown and has cavities which usually contain a mucilaginous substance.

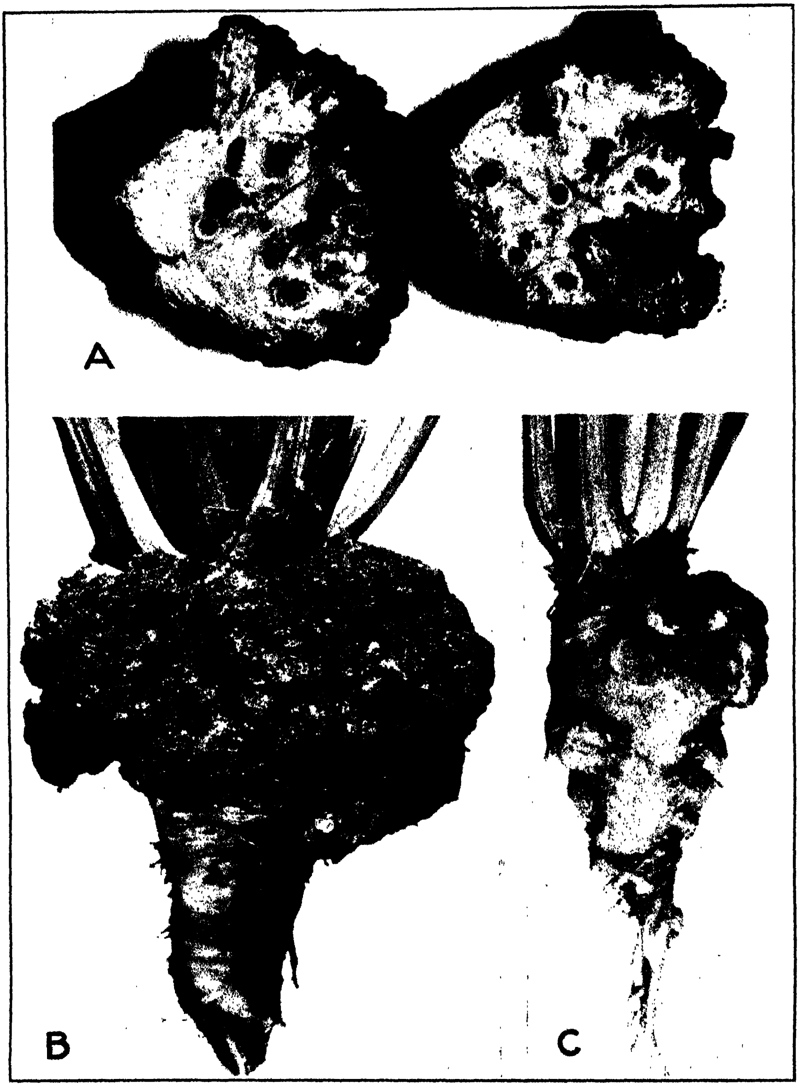

FIG. 4.—A, sugar beet cut across to show bacterial pockets in tubercles. Natural size. B, bacterial pocket disease of sugar beet produced by inoculating with a reisolation colony (the yellow-ringed type shown in pl. 1,B, *b*) Jan. 7, 1924. Photographed Feb. 26, 1924. Natural size. C, sugar beet inoculated with a white rubbery reisolation colony Jan. 31, 1924. Photographed Feb. 26, 1924. Natural size

THE CAUSAL ORGANISM

CONFUSION REGARDING ITS NAME

The organism causing the bacterial pocket disease was named *Bacterium beticolum* in 1911. In 1913 Serbinow [3] described an organism that he called *Bact. beticola*. This organism produces a so-called bacteriosis of sugar beets in which the whole root is gradually destroyed. There is no tubercle development, and the description of the organism producing the disease indicates that it is not the same as the one discussed in this paper.

Briefly, a few of the different characteristics of *Bact. beticola* Serb. are as follows:

> It appears on beef-peptone agar plates in 48 hours as a white round colony, becoming yellowish brown with a diffuse margin in three to five days; it develops gas in beef agar and gelatin when 2 per cent cane and 5 per cent grape sugar are added to them; it does not produce hydrogen sulphide or change nitrates to nitrites; its growth on potato cylinders is a muddy white, color hardly noticeable or entirely absent; it is peritrichous; it produces no capsules or chains; and neither the tissues of sugar beets infected by it nor the liquid from the tissues is slimy.

In 1915 Potebnia [4] discussed the names of both of these organisms. He stated that the name *Bacterium beticolum*, given to the bacterial pocket disease organism in 1911, is incorrect Latin and should be *Bact. beticola*. He knew that the name *Bact. beticola* had been used by Serbinow in 1913 to designate a different organism pathogenic to sugar beets, so he changed the name of the latter organism to *Bact. serbinowi* and that of *Bact. beticolum* to *Bact. beticola*. As Potebnia is right about the ending of the Latin word, this correction is accepted and the organism that produces tubercles on sugar beet should be called *Bact. beticola* (Smith, Brown, Townsend) Potebnia.

CULTURAL CHARACTERS

The beef agar, bouillon, and gelatin used in these experiments were made with beef infusion unless otherwise stated.

The colonies used in the cultural tests were all of the smooth type.

Beef-agar plates.—Colonies appear on the plates 21 to 28 hours after pouring from macerated gall tissue; they are cream colored to buff, 2 to 3 mm. in diameter, shining, mostly round and smooth (pl. 1, B, *a*), some round and wrinkled, and a few slightly lobed and either smooth or wrinkled. Many of the round smooth colonies are thin with a thickened place in the center and rather inconspicuous concentric rings. (Pl. 1, A.) In two to three days after the plates are poured, the colonies are yellow and 4 to 6 mm. in diameter, and the rings are still visible. There was an exception in the isolations from one lot of material as to initial color. The colonies came up white and were bluish in transmitted light. They became yellow in three days, however, although the shade of yellow was always light.

Beef-agar slants.—There is moderate filiform growth, flat, glistening, usually smooth, but often rugose on slants of beef agar with a pH of 6.8 to 7.1. It is yellow, translucent, viscid, and has practically no odor.

Beef bouillon.—Clouding occurs in 7 to 18 hours at a temperature of 25° C. in media with a pH range of 6.7 to 7.3. It is heavy in three to four days, a yellow ring is present, and strings of viscid growth hang down in the medium. There is also a thick growth at the bottom of the tube. Sometimes a yellow pellicle, which falls immediately on handling the tube, forms. In 11 days there is a yellow color throughout the medium. The sediment in the bottom is abundant and is viscid on agitation. The odor is not unpleasant. At the optimum temperature, 29° C., growth takes place in some of the colonies in three to four hours.

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[3] Serbinow, I. L. ÜBER DIE NEUE BAKTERIOSE DER ZUCKERRÜBENWURZEL. Zhur. "Boliezni Rastenif" 7: 237–258, illus. 1913. [In Russian. German résumé, p. 257–258.]

[4] Potebnia, A. A. [FUNGOUS PARASITES OF THE HIGHER PLANTS IN KHARKOV AND ADJACENT PROVINCES.] Kharkov Prov. Agr. Expt. Sta. 1: 27–29, illus. 1915. [In Russian. Reviewed by M. Shapovalov in Phytopathology 6: [293]–295. 1916.]

Whey-agar slants.—On whey agar with a pH of 6.8 to 7.4 the organism grows luxuriantly; some colonies have a smooth surface, others are rugose.

Potato cylinders.—At first there is a light-yellow growth, which changes to a deep yellow in less than a week. Three of the colonies produce a brighter yellow than the other three. The cylinders of all are slightly darkened. The diastasic action is slight.

Blood serum.—There is a fair amount of growth on Loeffler's blood serum. Some colonies produce a smooth surface, others a papillate surface growth. The color is a deep cream in some of the colonies and yellow in others. There is no liquefaction.

Bouillon over chloroform.—There is heavy clouding in 24 hours at 23° C. in tubes of beef bouillon of pH 6.8 containing 5 c. c. of chloroform.

Fermi's solution.—The medium is clouded in 3 days and a white pellicle is formed. In 6 days the pellicle is cream colored and festoons of growth hang from it into the medium; in 13 days the pellicles of all colonies are yellow and there is a yellow precipitate, which is rather fluffy.

Uschinsky's solution.—A pellicle (incomplete with some colonies) is formed in three days. There are strings and flakes of growth in the media of all colonies. The cultures were examined again in four months just before discarding, and the media of colonies 4, 5, and 6 were found to have changed to a brown or coffee color, clear, with a heavy, lighter colored, viscid precipitate, while the media of colonies 1, 2, and 3 were decidedly lemon colored, clear, with a light-yellow viscid precipitate. Because of doubt as to colonies 4, 5, and 6, plates were poured, and pure cultures of each appeared on the plates, but none of the many subcultures therefrom browned Uschinsky in 19 days.

Cohn's solution.—There is no growth of the organism in Cohn's solution.

PHYSIOLOGICAL CHARACTERS

Liquefaction of gelatin.—In beef-gelatin plates with a pH of 7.1 kept at a temperature of 20° to 22° C., the colonies are rounded up and thicker than on beef agar. There are two types, wrinkled and smooth, but all are round. The smooth ones are ringed, but they have fewer rings than those grown on agar. The colonies even on thinly sown plates are still small (3 mm.) after four days. Liquefaction begins in six to seven days. Thickly sown plates are entirely liquefied in 14 days.

Growth in gelatin stabs of pH 6.9 at 18° to 20° C. is best at the surface but occurs along the line of puncture. Liquefaction is usually crateriform; it begins in seven to eight days and occurs slowly across the surface. All the gelatin is liquefied in 22 to 30 days.

Hydrolysis of starch.—Plates were poured with beef-infusion agar, pH 8, containing 0.2 per cent of corn starch. When the agar had hardened, smears of the organism were made across the plates. After seven days there was a fair amount of growth, and the surfaces of some of the plates were flooded with iodine solution. There was no change in the color of the medium around the streak. Tests were made with the iodine again at 14 days. At this time on either side of the streak there was an area of 8 to 10 mm., which had a clear purplish tinge with little blue flecks in it, showing that there was very little destruction of starch. Fourteen plates were treated in this way. Plates of *Bacterium phaseoli* tested for comparison gave complete starch reduction in a broad band of 3 cm. on either side of the streak.

Toleration of sodium chloride.—The organism tolerates sodium chloride up to 9 per cent. In beef bouillon (pH 7.1 titrating+11) plus 9 per cent sodium chloride all six colonies used in these tests grew, but there was no growth with 9 per cent sodium chloride when the beef bouillon titrated 0 with a pH of 8.2.

Reduction of litmus.—In three days at the surface of litmus-milk cultures there is a trace of blue that later becomes pinkish (pale vinaceous lilac [5]). Reduction of litmus is complete in 20 to 30 days. Coagulation occurs in 10 to 20 days in five of the seven colonies; the casein is digested slowly, digestion being completed in about three months. Two infectious colonies did not coagulate milk and were slow in reducing the litmus.

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[5] Ridgway, R. Color standards and color nomenclature. 43 p., pl. XLIV. Washington, D. C., 1912.

Reduction of methylene blue.—All seven colonies tested decolorized methylene blue in milk in one day. Colonies 1, 2, 3, 4, and 7 coagulated it in 8 to 20 days.

Reduction of nitrates.—Nitrates are reduced to nitrites. Tests were made with nitrate-bouillon cultures 9 days old, using the starch-iodine-sulphuric acid test.

Indol production.—No indol is produced. Tests were made with six colonies grown in 1 per cent peptone water for 4 and 10 days. The sodium nitrite-sulphuric acid test was used. *Bacillus coli* grown as a check in some of the same medium and tried at the same time gave a positive test.

Hydrogen sulphide production.—The organism produces hydrogen sulphide. The different colonies grown on potato cylinders and in beef bouillon were tested by suspending lead-acetate paper in the culture tubes. The paper blackened in two to six days.

Ammonia production.—The organism produces ammonia. Both old and young cultures were tested with strips of filter paper moistened in Nessler's solution and suspended over the cultures. When the cultures were heated in a water bath, browning of the paper began immediately and reddish brown moisture ran down the sides of the tubes.

Gas production.—The organism is not a gas former. It was tested in fermentation tubes in the presence of the following carbon compounds: Saccharose, dextrose, lactose, maltose, glycerin, and mannit. A 1 per cent solution of each was made in a 1 per cent water solution of Difco peptone. There was no growth in the closed arm of the tube and no gas. Three colonies were tested. There was acid produced in all the solutions except that of lactose with colony 4. As some mistake was suspected, a new lactose solution was made up according to the formula used. It was inoculated and tested for pH after 18 days had elapsed, as in the first test. It was again alkaline. The pH readings are given in Table 1.

Table 1.—*Acid production by Bacterium beticola after a growth of 18 days in 1 per cent sugar solutions added to 1 per cent Difco peptone*

[Acidity indicated by pH readings]

Colonies	Chemicals						
	Glycerin	Dextrose	Saccharose	Lactose		Maltose	Mannit
				First test	Second test		
Check	6.6	6.4	6.8	6.8	6.4	6.6	6.8
Colony 1	6.2	4.6	6.2	6.2	5.4	5.8	5.4
Colony 2	6.4	3.7	4.7	5.8	----------	5.6	6.2
Colony 4	5.4	4.4	5.2	7.1	8.0	5.4	5.3

Optimum reaction for growth in beef bouillon and the titrable acidity and alkalinity.—The best growth in peptone beef-infusion bouillon takes place at pH 6.5 (+17), although the organism has a wide range and is not easily retarded, growing from pH 4.8 (+34) to pH 9.1 (−9). Good growth conditions are from pH 5.8 to 8.6 (+24 to −4). There is a fair amount of clouding at pH 5 (+32), only slight clouding at pH 4.8 (+34), and none at pH 4.5 (+37). Good clouding occurred at pH 8.2 to 8.6 (0 to −4), moderate at pH 9 (−8), weak at pH 9.1 (−9), and none at pH 9.5 (−13).

Tests were made to learn what changes were taking place in media of pH 9.1 and 6.6 after the organism had been growing 2, 5, 8, and 15 days. The results are shown in Table 2.

TABLE 2.—*Changes in the hydrogen-ion concentration of beef bouillon caused by the growth of Bacterium beticola in media near the optimum pH 6.6 and near the limit pH 9.1*

Number of days after inoculation	pH in bouillon of colony[a]—[Initial pH 9.1]			pH in bouillon of colony—[Initial pH 6.6]		
	No. 2	No. 4	No. 6	No. 2	No. 4	No. 6
2	8.0	7.8	8.0	6.2	6.4	6.4
5	8.0	7.8	8.0	6.2	6.4	6.4
8	8.2	8.2	8.2	6.8	6.8	6.8
15	8.2	8.2	8.2	6.8	6.8	6.8

[a] At the end of 5 days the pH of the check was 8.8.

TOLERATION OF ORGANIC ACIDS.—Tests were made with citric, oxalic, and tartaric acids by adding 0.1, 0.2, and 0.3 per cent of each to neutral beef bouillon. Growth was normal in the bouillon containing 0.1 and 0.2 per cent of the acids; and clouding occurred in that containing 0.3 per cent of citric acid, but there was no growth in the bouillons containing 0.3 per cent of either oxalic or tartaric acids. With 0.4 per cent citric acid there was no growth. The pH and titrable-acidity values are shown in Table 3.

TABLE 3.—*Toleration of organic acids by Bacterium beticola*

[Acids in beef bouillon]

Citric			Oxalic			Tartaric		
pH	Fuller's scale	Character of growth	pH	Fuller's scale	Character of growth	pH	Fuller's scale	Character of growth
7.1	+11	Heavy clouding in in 20 hours.	7.1	+11	Heavy clouding in 20 hours.	7.2	+10	Heavy clouding in 20 hours.
6.1	+21	Good clouding.	5.7	+25	Good clouding.	6.2	+20	Good clouding.
5.4	+28	Do.	4.9	+33	No clouding.	5.2	+30	No clouding.
5.1	+31	No clouding in 2 weeks.						

THERMAL RELATIONS.—The organism grows at temperatures of 1.5° to 39° C. Only two out of six colonies grew at 1.5°, but all grew at 39°. None grew at 40°. The optimum temperature is about 29°.

The thermal death point is between 51° and 52° C. when beef-bouillon cultures (pH 6.8 to 7.2) are exposed in a water bath for 10 minutes. When exposure was made at 50° good growth took place. In two tests six colonies grew at 51°, but none grew at 52°. At 51.5° all colonies grew in the first test, none in the second, and only one in the third.

OXYGEN RELATIONS.—For some time it was thought that *Bacterium beticola* was a facultative anaerobe. Numerous tests were made in which beef-bouillon and beef-agar transfers of pH 6.8 were placed in jars in which the oxygen was replaced by nitrogen or in specially devised jars from which the air was exhausted. In 18 hours there would be a faint growth, but this would not continue. When a medium less favorable for growth was used, such as agar of pH 8.9 to 9.1, in which development would not begin immediately, no growth occurred. It was therefore concluded that the organism was aerobic and that its apparent facultative anaerobic tendencies were due to the quick growth of the organism before the oxygen was replaced. As stated elsewhere, growth takes place in beef bouillon of pH 6.8 in three hours at a temperature of 29° C. with some of the colonies, which were infectious in the spring of 1925.

EFFECT OF FREEZING.—Transfers were made to beef bouillon of pH 6.7 from a 1-day-old culture, and after 15 minutes plates were poured to use for colony comparisons later. The transfers were then immersed in a mixture of cracked ice and salt, where they remained frozen solid for 20 minutes. They were thawed out quickly and more plates were poured. From 80 to 90 per cent of the colonies were killed by the freezing.

EFFECT OF DESICCATION.—The organism is somewhat resistant to drying. Sterile cover glasses receiving a drop of a 24-hour-old beef-bouillon culture were kept at room temperatures, 25° to 28.5° C. After the cultures had dried from 1 to 12 days the covers were dropped into tubes of beef bouillon. Growth took place in tubes receiving covers that had dried 7 days, but there was none in the 8 to 12 day tests. Plates were poured from the 7-day tubes and pure cultures of *Bacterium beticola* obtained.

LONGEVITY.—The organism lives for 14 months in sterile milk at room temperatures, 22° to 29° C. The milk may be dried to a jellylike consistency and the organisms remain alive. It lives from 5 to 8 months in agar and bouillon at room temperatures, and 13 to 16 months in beef bouillon if kept in the refrigerator at a temperature of 12° to 14° C.

GROWTH IN INDICATOR SUGAR AGAR.—Color changes were observed in beef agar of pH 6.7 to which 1 per cent dextrose, saccharose, galactose, lactose, and glycerin were added and the media colored with brom cresol purple, an indicator that becomes yellow in acid media. The organism grew rapidly on the sugars. The medium containing saccharose turned yellow in 48 hours without reddening; it became purple again in two weeks. The galactose and dextrose reddened in 18 hours, then turned yellow, beginning at the surface. In 42 hours the purple color returned just below the growth, and in two weeks the entire agar was purple. Lactose and glycerin became a faint greenish yellow color at the surface in 48 hours; this faint color was all through the agar in 72 hours. The next day the purple color returned to the surface, and in 7 days the lactose was purple again, but it took 15 days for the glycerin medium to become purple.

LITMUS AGARS.—The base medium consisted of 1 per cent Witte's peptone, 1 per cent agar, and litmus solution to which 1 per cent of the following sugars was added: Saccharose, dextrose, lactose, maltose, mannit, raffinose; also 0.5 per cent levulose and 0.5 per cent galactose.

In seven days there was slight reddening of the medium in saccharose, dextrose. maltose; a trace in glycerin; good reddening in mannit, galactose, and levulose, There was no reddening in raffinose or lactose. The litmus color returned after 2 days in levulose, maltose, and mannit, and in 5 to 7 days in galactose, dextrose, saccharose, and glycerin.

RELATION TO SUNLIGHT.—The organism is not very sensitive to sunlight. Thinly sown agar poured plates were exposed in bright sunlight at midday on bags of crushed ice out of doors, half of each plate being covered with black paper to serve as a check. An exposure of 65 minutes did not kill the organism, but one of 75 minutes did. One strain was able to withstand an exposure of 90 but not of 100 minutes.

COLOR PRODUCTION.—The color of the young bacterial growth is buff, but this quickly changes to a definite yellow. Yellow prevails in most media.

VIRULENCE.—Isolations made from Garden City and Rocky Ford beets in 1910 and 1912 and kept since at 12° to 15° C. and transferred several times a year were still virulent in January, 1924, after 12 and 14 years.

REACTION TO STAINS.—The organism stains readily with carbol fuchsine, gentian violet, and methyl violet. It is not acid fast and is Gram variable. This was determined by making repeated tests with the regular Gram's stain. Other Gram tests were made with 1-day agar cultures, using a mixture of carbol fuchsine, gentian violet, and buffers of different ranges (the Clark buffer series). The preparations were mordanted with Lugol's solution and decolorized with acetone. When the stain contained a buffer of high acid or high alkaline range, as pH 1 and 9, the test was positive. When the pH was about 5 and 6 the test was negative. This last test was suggested and carried out according to Stearn and Stearn's methods for testing the relation of acidity and alkalinity to Gram character,[6] and, as in their results, it seems that the hydrogen-ion concentration may play a rôle in determining the Gram character of an organism. As ordinarily tested, the organism is Gram negative.

EMENDED DESCRIPTION OF THE ORGANISM

Bacterium beticola is a short motile rod, usually paired, but it may occur singly, in clumps, or in chains of 6 to 10 elements. There are one to four flagella at either pole; these are long and in the paired rods give the appearance of peritrichous flagella. The size of the rod is 0.6 to 2 µ long, 0.4 to 0.8 µ wide; irregular forms of a round or oval shape twice the ordinary size were noted. Capsules are produced, but no spores. The organism is Gram variable; not acid fast; aerobic; color yellow; liquefies gelatin slowly, but not Loeffler's blood serum; reduces nitrates; produces hydrogen sulphide and ammonia but not indol; has weak diastasic action; coagulates milk; reduces litmus in 10 to 30 days, the litmus being first blued; forms acid from dextrose, saccharose, maltose, and mannit, but not lactose; does not produce gas; grows well in Uschinsky's and Fermi's solutions, making them viscid, but not at all in Cohn's; optimum temperature about 29° C., maximum 39°, minimum 1.5°; thermal death point 51° to 52°; not sensitive to sodium chloride, tolerating 9 per cent in beef bouillon; has a pH range of 4.8 to 9.1 (+34 to −9); not killed readily by drying or exposure to sunlight; lives 14 months in sterile milk at 22° to 29°; stains readily with gentian violet, methyl violet, and carbol fuchsine; pathogenic to sugar and garden beets, producing tubercles with cavities.

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[6] STEARN, E. W., and STEARN, A. E. THE CHEMICAL MECHANISM OF BACTERIAL BEHAVIOR. I. BEHAVIOR TOWARD DYES—FACTORS CONTROLLING THE GRAM REACTION. Jour. Bact. 9: 463-477, illus. 1924.

The index number based on the chart of the Society of American Bacteriologists [7] is 502 var.-31125-1222.

NATURAL INFECTION AND CONTROL

The organism is a wound parasite, which probably gets into the sugar beet through cultivation wounds or through breaks in the tiny rootlets made when the beets are thinned. The disease occurs in fields heavily manured or in those that have received large quantities of sodium or potassium nitrate. Up to the present it has not been known to occur under other conditions. The control therefore is rather easy and obvious.

SUMMARY

A gall disease of sugar beets in the nature of tubercle outgrowths with pockets is described in this paper. It is produced by an organism to which the name *Bacterium beticola* has been given (corrected from *Bact. beticolum*). The organism is a wound parasite, which stimulates the tubercles to form, discolors the gall tissue, and produces cavities, which usually contain a viscid fluid. The tubercles with pockets and exudate were reproduced on sugar beets and garden beets by inoculation.

In outward appearance the disease frequently resembled crown gall but can easily be distinguished from it by cutting through the outgrowth and noting whether or not there are pockets and stained tissue within. In crown gall the tissue is white and sound.

The disease so far as known occurs only in soil rich in nitrogenous fertilizers, and on this account its control is not difficult. It is known to occur in Kansas, Colorado, and Virginia.

The parasite is a yellow, polar flagellate, and is not known to produce tubercles on any other plants than sugar and garden beets. The index number is 502 var.-31125-1222.

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[7] SOCIETY OF AMERICAN BACTERIOLOGISTS. DESCRIPTIVE CHART. [2] p. [1924.

GROWTH AND SENESCENCE IN RED DANISH COWS AS MEASURED BY THE RATE OF MILK SECRETION[1]

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INTRODUCTION

A recent paper by Davidson (*5*)[2] introduces a new form of growth and senescence equation, which he has developed as a result of an analysis of certain records of live weight and milk yield of purebred Jersey cows. Davidson's equation is of the form

$$\log Y = a - be^{-kt} - ce^{ht} \qquad (1)$$

in which Y is the rate of milk yield at the time or age t. The derivation of the equation is fully explained in the reference given. It is sufficient here to say that k represents the rate of decrease in growth power of the body cells, while h represents the rate of loss in physiological activity of the cells. The constants b and c are said to locate the curve in time; that is, their values depend upon the time origin but are independent of the time unit. The values of k and h vary directly with the time unit but are independent of the time origin.

The value of any equation of this kind can be fully developed only by its application to observed facts. As mentioned, Davidson has applied it successfully to certain records of the Jersey breed. It is applied in the present paper to somewhat similar milk-yield data of the Red Danish breed.

While t in the equation may be measured in any unit from any origin, it is suggested in the interest of uniformity, that t be measured in years from birth, being thus a direct measure of age in years. It seems desirable also to measure milk yield on an energy basis in order to have directly comparable values so far as variability in composition of the milk is concerned. This basis of measuring milk yield was suggested by Gaines and Davidson (*8*) and has recently been more fully elaborated (*7*).

SOURCE AND TREATMENT OF DATA

The cooperating Danish agricultural societies (*12*) publish a herdbook of cows of the Red Danish dairy breed, to which are admitted cows of that breed which meet certain requirements. The essential feature of the entrance requirements pertains to the milk production of the cow. It is required that the cow shall produce at least 160 kgm. of "butter" (about 315 pounds of fat) as an average per year for at least three years, and shall have an average fat percentage of not less than 3.60. However, cows having an average fat percentage of 3.45 to 3.60 may be admitted on the same general terms, except that the average yield shall be not less than 175 kgm. of "butter" (about 345

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[1] Received for publication May 28, 1928; issued October, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 180.

pounds of fat). In some respects this system is like the American system of advanced registry, but since the entrance requirements are based on the yield over a period of years there is no object of prolonging lactation by delayed breeding and the cows are bred to freshen every year. It should be noted that this breeding feature is in line with economical production (*6*, *13*) and seems to be a requirement that might well be emulated by advanced registries in this country.

The "butter" yield is computed from the weight of milk and its percentage fat content by the formula $B=\frac{M(f-0.15)}{86}$ where B is butter and M is milk, both in kilograms, and f is fat percentage.

The herdbooks include, in addition to the yearly records of the cow herself, also the yearly records of her female ancestors so far as available. The yearly records are for the fiscal year beginning October 1, and include the milk and butter yields. Any fiscal-year record which is considered by the authorities in charge of the herdbooks to be not representative is inclosed in brackets and excluded from the average. The published records include also the date of birth of the animals.

The data of the present paper are taken from the records of the ancestors of the herdbook cows entered in volumes 1, 2, and 3. The records of the herdbook cows themselves are not used for the reason that the entrance requirements must exclude a certain proportion of the population. This selective effect would apply also to their ancestors, but in a much smaller degree. The records of all ancestors so far as reported have been used, but bracketed yearly records have been excluded.

The milk and butter records were extracted in four main groups according to the age of the cow in years at a date 1.5 months following the beginning of the fiscal year, viz, Group 1, 1 to 1.25, 2 to 2.25, etc.; Group 2, 1.25 to 1.5, 2.25 to 2.5, etc.; Group 3, 1.5 to 1.75, 2.5 to 2.75, etc.; Group 4, 1.75 to 2, 2.75 to 3, etc. The total milk and butter yield of each age class was then computed by direct summation of the yearly records excluding bracketed records. From these totals were computed the average fat percentages and energy yields by use of the formulas, $f=86B/M+0.15$, where f is the average fat percentage; and $F.\ C.\ M.=\frac{0.9314M+28.439B}{N}$, where N is the number of records, M is milk in kilograms, B is butter in kilograms, and $F.\ C.\ M.$ is energy value in terms of pounds of 4 per cent milk. The $F.\ C.\ M.$ estimate is the equivalent of the usual $0.4M+15F$ formula (*8*), converting to the English unit. The only excuse for conversion to the English unit is to make the records directly comparable with records in this country so far as the unit of weight is concerned. The results of the above computations are shown graphically in Figure 1.

The frequency distributions of Figure 1 show an orderly irregularity and seem to mean that there is a pronounced tendency for the Danish dairyman to have his cows freshen during September and October.

The fat percentage curve for the most part lies between 3.7 and 3.8, and evidences a tendency to decrease very slightly with age. As above noted, certain requirements are enforced with reference to the fat percentage of the herdbook cows and these requirements are evidently rather rigorous. In the introduction to volume 1 is given a summary (*12, Bd. 1, p. XXI, Table 10*) which shows the average fat percentage of all cows tested as 3.36 in 1900, and this has gradually increased to 3.66 in 1920. It is evident, therefore, that the herdbook requirement as to fat percentage selects not only the higher testing individuals, but also higher testing mothers, all of which may be regarded as evidence that the fat percentage character is heritable and subject to modification by selective mating.

The *F. C. M.* or energy yield curve is the one of principal interest from the point of view of this paper. It shows the familiar, rapidly rising values up to 6 years, fairly stationary values from 6 to 11 years, with a descending tendency after that age. The work is next to fit equation (1) to these energy yield data of Figure 1.

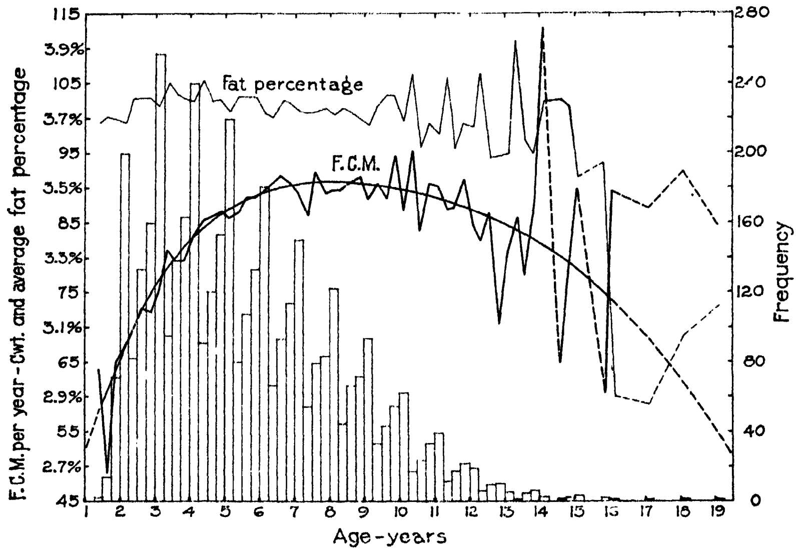

FIG. 1.—Relation of age to energy yield in Red Danish cows. Age is computed to a date 1.5 months following the beginning of the fiscal year. The energy yield is for the fiscal year and is expressed in terms of fat-corrected milk (*F. C. M.*) or 4 per cent milk. One pound *F. C. M.*=340 large calories. The smooth curve is that of Figure 2. The fat percentage shown by the upper lighter line curve is a weighted average. The numbers of records at the age classes are shown by the columns. There are 4,109 annual records of 740 different cows, an average of 5.55 years for each cow

FITTING THE EQUATION

In the first place, it seems desirable to try to eliminate some of the sharp irregularities of the *F. C. M.* curve of Figure 1 by the use of a longer age interval, say 6 or 12 months. The groups of Figure 1 have been, accordingly, combined into a new grouping, viz, 1.5 years (1.25 to 1.75), 2.0 years (1.75 to 2.25), ------ 5.5 years (5.25 to 5.75), 6.0 years (5.5 to 6.5), 7.0 years (6.5 to 7.5), -------. The new grouping involves a duplication of the class at 5.5 to 5.75 (107 records), which group now appears in both the 5.5 and 6.0 year classes. These new age values, 1.5, 2.0, 2.5, etc., are to constitute the values of t in equation (1).

The corresponding mean *F. C. M.* values are shown numerically in Table 1, column Y, and graphically in Figure 2. From them are to be derived the constants of equation (1). It will be noted that the last three observations of Figure 2 are out of line with the trend of the other values. These observations are for one and the same cow (mother's mother's mother of No. 1481), and since they seem to represent a very unusual animal they are excluded in fitting the equation.

For the purpose of fitting equation (1) to the data of Table 1 it is convenient to write it,

$$y = a - be^{-kx} - ce^{hx} \qquad (2)$$

in which $y = \log Y$ and x is time in units of six months with origin at the first observation, $x = 2t - 3$. The constants of equation (2) may be readily transformed to those of equation (1). The equation is not

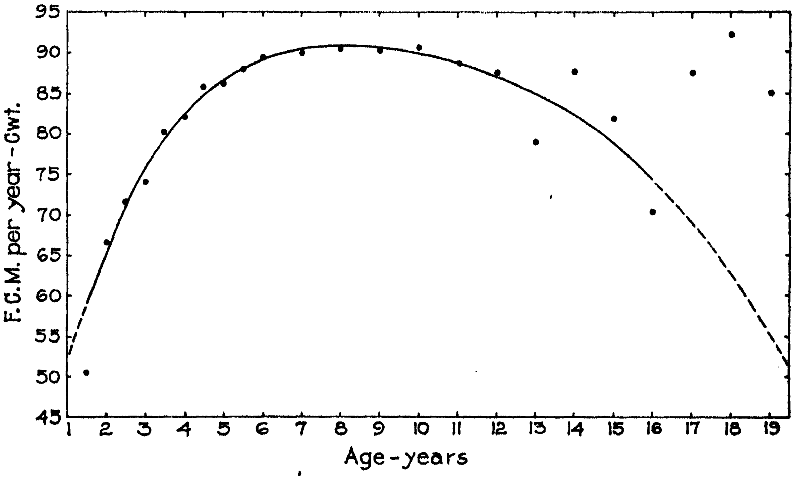

FIG. 2.—Relation of age to energy yield in Red Danish cows. The data are the same as those of Figure 1, combined in coarser age groups. The smooth curve has been fitted to the observations (except the last three) by the method of least squares and giving weight to the frequencies. Its equation is,

$$\log F.\ C.\ M.\ (\text{pounds}) = 3.977502 - 0.437979e^{-0.505140t} - 0.00156870e^{0.263274t}$$

in which t is age in years, origin at birth, and reckoned to a date 1.5 months following the beginning of the fiscal year

adapted to the application of direct algebraic methods. A first approximation of the constants may be made by graphic methods and these values then corrected by the method of least squares.

For the first-approximation constants the same symbols may be used in the upper case, $y_1 = A - Be^{-Kx} - Ce^{Hx}$. In data of the present nature the last term may be neglected at low values of x, and the second term may be neglected at high values of x. For low values of x we may write $A - y = Be^{-Kx}$ or $\log (A - y) = \log B - 0.4343Kx$. Likewise, for high values of x, $\log (A - y) = \log C + 0.4343Hx$. It is not difficult to guess at a value for A which is near its rightful value. From Figure 2 one may guess that if the left-hand portion of the curve were continued without the depressing influence of the last term, it would reach a value of 9,400 to 9,500, say 9,450 pounds. The logarithm of 9,450 is 3.975 and we may assume $A = 3.975$.

TABLE 1.—*Age changes in the rate of milk secretion of Red Danish Cows* [a]

t	x	n	f	Y	y	y_1	y_2	Y_2	ΔY
1.5	0	16	3.70	5,060	3.70415	3.75408	3.76988	5,887	−827
2.0	1	271	3.69	6,654	3.82308	3.80241	3.81537	6,537	+117
2.5	2	215	3.76	7,163	3.85509	3.83990	3.85059	7,089	+74
3.0	3	417	3.74	7,409	3.86976	3.86895	3.87782	7,548	−139
3.5	4	233	3.78	8,009	3.90358	3.89141	3.89881	7,922	+87
4.0	5	403	3.75	8,189	3.91323	3.90872	3.91494	8,221	−32
4.5	6	211	3.78	8,569	3.93293	3.92201	3.92727	8,458	+111
5.0	7	373	3.73	8,607	3.93485	3.93213	3.93661	8,642	−35
5.5	8	187	3.76	8,787	3.94384	3.93076	3.94361	8,782	+5
6.0	9	494	3.73	8,931	3.95090	3.94541	3.94875	8,887	+44
7.0	11	410	3.73	8,975	3.95303	3.95229	3.95481	9,012	−37
8.0	13	327	3.72	9,035	3.95593	3.95492	3.95691	9,056	−21
9.0	15	264	3.70	9,011	3.95477	3.95448	3.95609	9,038	−27
10.0	17	176	3.75	9,050	3.95665	3.95153	3,95288	8,972	+78
11.0	19	106	3.67	8,856	3.94724	3.94621	3.94742	8,860	−4
12.0	21	63	3.67	8,726	3,94082	3.93831	3.93954	8,700	+26
13.0	23	25	3.60	7,876	3.89631	3.92736	3.92881	8,488	−612
14.0	25	13	3.64	8,750	3.94201	3.91258	3.91458	8,215	+535
15.0	27	6	3.62	8,180	3.91275	3.89286	3.89589	7,868	+312
16.0	29	3	3.27	7,020	3.84634	3.86672	3.87146	7,438	−418
17.0	-----	1	2.88	8,727	-----	-----	3.83962	6,912	-----
18.0	-----	1	3.08	9,210	-----	-----	3.79815	6,283	-----
19.0	-----	1	3.16	8,479	-----	-----	3.74417	5,548	-----

[a] t = Age in years, origin at birth.
$x = 2t - 3$ = Age in units of 6 months, origin at $t = 1.5$.
n = Number of records.
f = Average fat percentage.
Y = Average energy yield in terms of pounds of 4-per-cent milk.
$y = \log_{10}$ of Y.
y_1 = Calculated y by first-approximation equation.
y_2 = Calculated y by second-approximation equation.
Y_2 = Calculated energy yield by second-approximation equation, pounds of 4-per-cent milk.
$\Delta Y = Y - Y_2$ = Observed minus calculated energy yield, pounds of 4-per-cent milk.

The next step is to plot log $(A-y)$ against x. This is done in Figure 3 and the two straight lines are drawn to fit the plotted values as well as may be by visual inspection. The K line intercepts the y axis at $\bar{1}.34$ and this is log B, consequently $B = 0.219$. It cuts the ordinate at $x = 10$ at $\bar{2}.25$, that is, $-0.4343K = \frac{\bar{2}.25 - \bar{1}.34}{10}$, from which $K = 0.251$. If A is chosen grossly too large or too small this may be detected in a systematic curvature of the plot values. This is the test used by Brody (*1, Fig. 3*), but it is not a very sensitive test.

The observations at the higher values of x, Figure 3, are rather equivocal for determination of the H line. The line drawn cuts the ordinate at $x = 16$ at $\bar{2}.25$, and at $x = 28$, at $\bar{2}.975$. Consequently, $0.4343H = \frac{\bar{2}.975 - \bar{2}.25}{28 - 16}$, and $H = 0.139$. If this line is projected to the left it intercepts the y axis at $\bar{2}.25 - 16\,\frac{\bar{2}.975 - \bar{2}.25}{12} = \bar{3}.283$. That is, log $C = \bar{3}.283$, and $C = 0.00192$.

We have therefore as a first approximation, $y_1 = 3.975 - 0.219e^{-0.251x} - 0.00192e^{0.139x}$. The values calculated from this equation are shown in the seventh column of Table 1. These values are derived at once by the use of a computing machine by negative summation of the products $B{\cdot}2$ and $C{\cdot}3$ with A, where 2 and 3 refer to the values in these columns of Table 2. The differences, Δy_1, are given in the last column of Table 2. They are the observed values minus the calculated values, $y - y_1$.

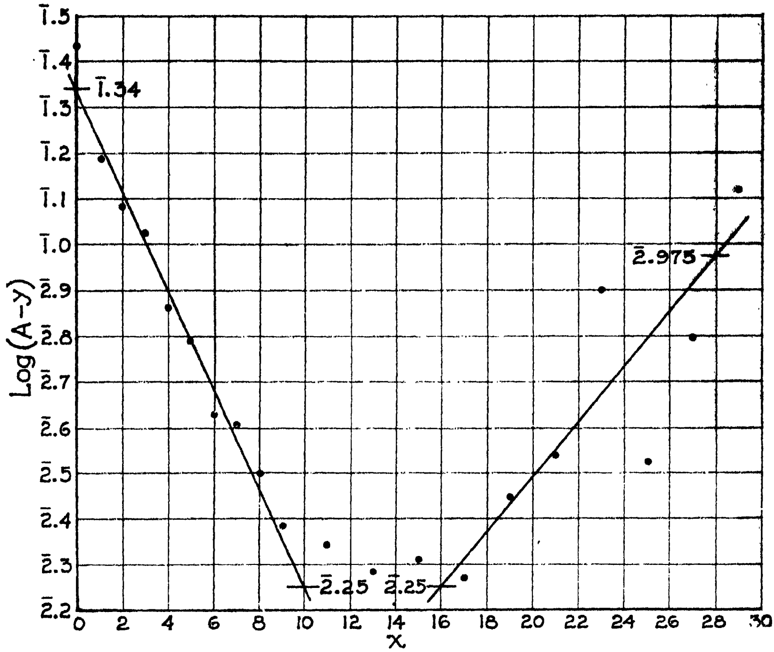

Fig. 3.—Graphic determination of the first-approximation constants

The next step is to derive corrections to the approximate constants, which we may designate ΔA, ΔB, etc., such that $(A+\Delta A)=a$, $(B+\Delta B)=b$, etc., where a, b, etc., are the correct values by the least-squares criterion. The details of the calculus of this process are given in various texts (*11, Chap. VI*). This leads to a new series of 20 observation equations of the form

$$\Delta A - e^{-Kx}\Delta B - e^{Hx}\Delta C + Bxe^{-Kx}\Delta K - Cxe^{Hx}\Delta H = \Delta y_1$$

The normal equations of these new observation equations are as follows:

$$+(\Sigma n)\Delta A - (\Sigma ne^{-Kx})\Delta B - (\Sigma ne^{Hx})\Delta C + B(\Sigma nxe^{-Kx})\Delta K - C\,(\Sigma nxe^{Hx})\Delta H = +\Sigma n\Delta y_1$$

$$-(\Sigma ne^{-Kx})\Delta A + [\Sigma n(e^{-Kx})^2]\Delta B + (\Sigma ne^{-Kx}e^{Hx})\Delta C - B[\Sigma nx(e^{-Kx})^2]\Delta K + C(\Sigma nxe^{-Kx}e^{Hx})\Delta H = -\Sigma ne^{-Kx}\Delta y_1.$$

$$-(\Sigma ne^{Hx})\Delta A + (\Sigma ne^{-Kx}e^{Hx})\Delta B + [\Sigma n(e^{Hx})^2]\Delta C - B(\Sigma nxe^{-Kx}e^{Hx})\Delta K + C[\Sigma nx(e^{Hx})^2]\Delta H = -\Sigma ne^{Hx}\Delta y_1.$$

$$+B(\Sigma nxe^{-Kx})\Delta A - B[\Sigma nx(e^{-Kx})^2]\Delta B - B(\Sigma nxe^{-Kx}e^{Hx})\Delta C + B^2[\Sigma nx^2(e^{-Kx2})]\Delta K - BC(\Sigma nx^2e^{-Kx}e^{Hx})\Delta H = +B\Sigma nxe^{-Kx}\Delta y_1.$$

$$-C(\Sigma nxe^{Hx})\Delta A + C(\Sigma nxe^{-Kx}e^{Hx})\Delta B + C[\Sigma nx(e^{Hx})^2]\Delta C - BC(\Sigma nx^2e^{-Kx}e^{Hx})\Delta K + C^2[\Sigma nx^2(e^{Hx})^2]\Delta H = -C\Sigma nxe^{Hx}\Delta y_1$$

The factor n is introduced in the normal equations to give weight to the number of records represented at each value of x. This is equivalent to weighting or multiplying each observation equation by the square root of its n. Since the standard error of the means represented by each of the observation equations should vary inversely as $\sqrt{n}$ this seems to be a fair method of weighting.

For the computation of the numerical coefficients in the normal equations the work may be arranged as in Table 2. The values in column 2 of Table 2 may be obtained by inserting the constant factor $e^{-0.251}=0.7780224$ in the computing machine and multiplying successively by the last obtained value as recorded. At $x=9$ the factor is changed to $e^{-0.502}=0.6053188$. The final result at $x=29$ is independently computed by the use of logarithms, and agreement of the results checks the entire column. A similar procedure is followed for column 3. All of the coefficients are obtainable directly from Table 2 either by summation of the columns or by summation of the products of pairs of columns, by the use of a computing machine. If it is not desired to take account of the differences in the number of records contributing to the mean observations, columns 6, 7, 8, 9, and 10 of Table 2 will be omitted, thus considerably reducing the work of computation. That is to say, the values of n in column 6 are taken as unity throughout, and consequently the values of columns 7, 8, 9, and 10 become identical with those of columns 2, 3, 4, and 5, respectively. Where the observed mean values are fairly regular, this shortened procedure may be fully justified.

TABLE 2.—*Data for computation of numerical coefficients of the normal equations*

x	2 e^{-Kx}	3 e^{Hx}	4 xe^{-Kx}	5 xe^{Hx}	6 n	7 ne^{-Kx}	8 ne^{Hx}	9 nxe^{-Kx}	10 nxe^{Hx}	11 Δy_1
0	1.00000	1.0000	0	0	16	16.00000	16.0000	0	0	−0.04993
1	.77802	1.1491	.77802	1.1491	271	210.84342	311.4061	210.84342	311.4061	+.02067
2	.60532	1.3205	1.21064	2.6410	215	130.14380	283.9075	260.28760	567.8150	+.01519
3	.47095	1.5174	1.41285	4.5522	417	196.38615	632.7558	589.15845	1,898.2674	+.00081
4	.36641	1.7437	1.46564	6.9748	233	85.37353	406.2821	341.49412	1,625.1284	+.01217
5	.28508	2.0037	1.42540	10.0185	403	114.88724	807.4911	574.43620	4,037.4555	+.00451
6	.22180	2.3025	1.33080	13.8150	211	46.79980	485.8275	280.79880	2,914.9650	+.01092
7	.17257	2.6459	1.20799	18.5213	373	64.36861	986.9207	450.58027	6,908.4449	+.00272
8	.13426	3.0405	1.07408	24.3240	187	25.10662	568.5735	200.85296	4,548.5880	+.00408
9	.10446	3.4939	.94014	31.4451	494	51.60324	1,725.9866	464.42916	15,533.8794	+.00549
11	.06323	4.6136	.69553	50.7496	410	25.92430	1,891.5760	285.16730	20,807.3360	+.00074
13	.03827	6.0922	.49751	79.1986	327	12.51429	1,992.1494	162.68577	25,897.9422	+.00101
15	.02317	8.0447	.34755	120.6705	264	6.11688	2,123.8008	91.75320	31,857.0120	+.00029
17	.01403	10.6229	.23851	180.5893	176	2.46928	1,869.6304	41.97776	31,783.7168	+.00512
19	.00849	14.0274	.16131	266.5206	106	.89994	1,486.9044	17.09886	28,251.1836	+.00103
21	.00514	18.5230	.10794	388.9830	63	.32382	1,166.9490	6.80022	24,505.9290	+.00251
23	.00311	24.4594	.07153	562.5662	25	.07775	611.4850	1.78825	14,064.1550	−.03105
25	.00188	32.2983	.04700	807.4575	13	.02444	419.8779	.61100	10,496.9475	+.02943
27	.00114	42.6495	.03078	1,151.5365	6	.00684	255.8970	.18468	6,909.2190	+.01989
29	.00069	56.3181	.02001	1,633.2249	3	.00207	168.9543	.06003	4,899.6747	−.02038

The following checks assure the accuracy of the arithmetic up to this point except as to column 11 $\Sigma 2\cdot 8=\Sigma 3\cdot 7=1934.904089057$; $\Sigma 4\cdot 8=\Sigma 5\cdot 7=11{,}229.890843528$; $\Sigma 5\cdot 8=\Sigma 3\cdot 10=3{,}059{,}557.16958729$; $\Sigma 5\cdot 9=\Sigma 4\cdot 10=98{,}484.338274958$.

Column 11 is derived from Table 1, $\Delta y_1=y-y_1$

The normal equations in terms of Table 2 are:

$$
\begin{array}{cccccl}
\Delta A & \Delta B & \Delta C & \Delta K & \Delta H & \text{Absolute terms} \\
+\Sigma 6 & -\Sigma 2\cdot 6- & \Sigma 3\cdot 6+ & B\Sigma 4\cdot 6- & C\Sigma 5\cdot 6 & =+\Sigma 6\cdot 11 \\
-\Sigma 2\cdot 6+ & \Sigma 2\cdot 7+ & \Sigma 3\cdot 7- & B\Sigma 4\cdot 7+ & C\Sigma 5\cdot 7 & =-\Sigma 7\cdot 11 \\
-\Sigma 3\cdot 6+ & \Sigma 3\cdot 7+ & \Sigma 3\cdot 8- & B\Sigma 4\cdot 8+ & C\Sigma 5\cdot 8 & =-\Sigma 8\cdot 11 \\
+B\Sigma 4\cdot 6- & B\Sigma 4\cdot 7- & B\Sigma 4\cdot 8+ & B^2\Sigma 4\cdot 9- & BC\Sigma 5\cdot 9 & =+B\Sigma 9\cdot 11 \\
-C\Sigma 5\cdot 6+ & C\Sigma 5\cdot 7+ & C\Sigma 5\cdot 8- & BC\Sigma 5\cdot 9+ & C^2\Sigma 5\cdot 10 & =-C\Sigma 10\cdot 11
\end{array}
$$

From Table 2:

$$\begin{array}{llll}
\Sigma 6 = 4,213. & & & \\
\Sigma 2\cdot 6 = 989.87202 & & & \\
\Sigma 3\cdot 6 = 18,212.3751 & & & \\
\Sigma 4\cdot 6 = 3,981.00805 & \text{and} & B\Sigma 4\cdot 6 = & 871.84077 \\
\Sigma 5\cdot 6 = 237,819.0655 & \text{and} & C\Sigma 5\cdot 6 = & 456.61261 \\
\Sigma 6\cdot 11 = 21.39635 & & & \\
\Sigma 2\cdot 7 = 447.89453 & & & \\
\Sigma 3\cdot 7 = 1,934.9041 & & & \\
\Sigma 4\cdot 7 = 1,130.62666 & \text{and} & B\Sigma 4\cdot 7 = & 247.60725 \\
\Sigma 5\cdot 7 = 11,229.8908 & \text{and} & C\Sigma 5\cdot 7 = & 21.56139 \\
\Sigma 7\cdot 11 = 8.3705999 & & & \\
\Sigma 3\cdot 8 = 164,753.91 & & & \\
\Sigma 4\cdot 8 = 11,229.8908 & \text{and} & B\Sigma 4\cdot 8 = & 2,459.3461 \\
\Sigma 5\cdot 8 = 3,059,557.2 & \text{and} & C\Sigma 5\cdot 8 = & 5,874.3498 \\
\Sigma 8\cdot 11 = 51.912017 & & & \\
\Sigma 4\cdot 9 = 4,526.0373 & \text{and} & B^2\Sigma 4\cdot 9 = & 217.07327 \\
\Sigma 5\cdot 9 = 98,484.338 & \text{and} & BC\Sigma 5\cdot 9 = & 41.410694 \\
\Sigma 9\cdot 11 = 23.8133664 & \text{and} & B\Sigma 9\cdot 11 = & 5.2151272 \\
\Sigma 5\cdot 10 = 62,928,914. & \text{and} & C^2\Sigma 5\cdot 10 = & 231.98115 \\
\Sigma 10\cdot 11 = 422.98409 & \text{and} & C\Sigma 10\cdot 11 = & .8121294
\end{array}$$

And hence the normal equations are:

$$\begin{array}{l}
+4213.\ \Delta A - 989.87202\Delta B - 18,212.3751\Delta C + 871.84077\,\Delta K \\
\qquad - 456.61261\Delta H = +21.39635 \\
-989.87202\ \Delta A + 447.89453\Delta B + 1,934.9041\Delta C - 247.60725\,\Delta K \\
\qquad + 21.56139\Delta H = -8.3705999 \\
-18,212.3751\Delta A + 1,934.9041\Delta B + 164,753.91\Delta C - 2,459.3461\Delta K \\
\qquad + 5,874.3498\Delta H = -51..912017 \\
+871.84077\ \Delta A - 247.60725\Delta B - 2,459.3461\ \Delta C + 217.07327\,\Delta K \\
\qquad - 41.410694\Delta H = +5.2151272 \\
-456.61261\ \Delta A + 21.56139\ \Delta B + 5,874.3498\ \Delta C - 41.410694\,\Delta K \\
\qquad + 231.98115\Delta H = -0.8121294
\end{array}$$

By solution of these equations we have:

$$\begin{aligned}
\Delta A &= +0.002502 \\
\Delta B &= -0.013702 \\
\Delta C &= +0.00040833 \\
\Delta K &= +0.001570 \\
\Delta H &= -0.007363
\end{aligned}$$

and applying these corrections to the first-approximation constants, remembering that $a = (A + \Delta A)$, $b = (B + \Delta B)$, etc., we have the second-approximation equation:

$$y_2 = 3.977502 - 0.205298e^{-0.252570x} - 0.00232833e^{0.131637x}$$

In order to convert this to read reckoning time in years with origin at birth, K and H are multiplied by 2, while $b = 0.205298/e^{-3(0.252570)} = 0.437979$ and $c = 0.00232833/e^{3(0.131637)} = 0.00156870$.

The final equation is therefore:

$$\log F.\ C.\ M. = 3.977502 - 0.437979e^{-0.505140t} - 0.00156870e^{0.263274t}$$

the curve of which is shown in Figures 1 and 2. The equation reads in pounds. To make it read in kilograms it is necessary merely to subtract 0.343337 from the a constant (1 pound = 0.45359 kgm. and log 0.45359 = −0.343337).

DISCUSSION

The measurement of growth and senescence by the quantity of milk yielded during various years of the cow's life may be regarded as a measurement of vital activity at the corresponding ages. Since milk secretion is very profoundly connected with reproduction and in the natural course of life is manifest periodically for a limited time following parturition, there are obvious grounds for measuring age of the cow at the date of calving, and for measuring the rate of milk secretion at its flush a few days after calving. From this point of view, the seven-day records of the Holstein breed have much merit.

There is also good reason for measuring the rate of milk secretion by the yield over a longer period, since we then consider both the rate of yield at the start of lactation and the rate of decline with advance in lactation. The yearly yield is a composite measurement, and clearly any thorough analysis of the problem should consider separately the two items, initial rate of yield and rate of decrease in rate of yield.

The above considerations are not completely satisfied by the present data. They deal with successive 12-months periods without regard to the position of the point at which calving occurs in the periods, and age is computed to a date 1.5 months following the beginning of the period.

A point of interest in connection with the fitted curve of equation (1) is the age of maximum yield. From the first derivative of the equation, the curve reaches a maximum when $t=\frac{\log \frac{bk}{ch}}{0.4343(k+h)}$, the constants being taken as positive values. From the final values of the constants the maximum is reached when $t=8.18$. By computing age to the middle of the periods, the maximum is reached at 8.6 year of age.

From either Figure 1 or Figure 2 it is apparent that equation (1) as fitted conforms very well to the trend of the observations. The observation at 1.5 years, however, is quite far below the calculated value. On turning back to the 16 original records from which this observation is derived, it was found that several of them were for less than 250 days. It is quite probable that this observation does not fully represent the yield to be expected of cows of this age because of the inclusion of heifers which calved for the first time so late in the fiscal year as to make the time in milk abnormally short.

The various types of equations that have been used by different investigators to express the relation between age and milk yield are of interest. Some of them are:

	Author	Equation
(I)	Pearl (*9*)	$y=a+bx+cx^2+d\log x$
(II)	Clark (*4*)	$y=a+bx+cx^2+dx^3$
(III)	Sanders (*14*)	$\log y=a+bx+cx^2+dx^3$
(IV)	Brody et al. (*3*)	$y=ae^{-kx}-be^{-hx}$
(V)	Brody (*2*)	$y=a-be^{-kx}$
(VI)	Davidson [3]	$y=a-be^{-kx}-cc^{hx}$
(VII)	Davidson (*5*)	$\log y=a-be^{-kx}-ce^{hx}$

The "logarithmic" equation of Pearl was the first of these to be put forward, and it has been used quite extensively. There is a good deal of enchantment in the very word "logarithmic" and the equation is an adaptable one, *provided the x origin is shrewdly chosen.* The log term disappears entirely at $x=1$; and at $x=0$ it follows that $y=-\infty$. At very low values of x the log term changes very rapidly with change in x.

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[3] Unpublished. This is an obvious modification of (V). That is, the modification is obvious enough after Davidson's work.

It has been pointed out by Brody (*2*) and Davidson that Pearl's equation is a purely empirical one.

The equations of Clark and Sanders seem to belong also in the class of empirical equations. Their constants are not susceptible of any interpretation of physical significance.

The equation (IV) of Brody et al. was introduced to test the possibility of interpreting the change in milk yield with age as a function of some chemical reaction, the general course of which has been determined by the physical chemist. The constants k and h are velocity constants. The equation was fitted successfully to seven-day Holstein records but did not seem to conform very well with the observed values for yearly yields. Just why the equation should apply in the case of seven-day records and not in the case of longer-time records is not clear.

Brody has later applied his growth equation (V), $y=a-be^{-kx}$, to a wide assortment of age and milk-yield data, up to the age of maximum production. This equation, of course, does not apply to the decreasing rates of yield which come at advanced ages.

Equation (VII) is that of Davidson, which is used in the present paper. Davidson has employed (but not published) a modification of Brody's equation. This modified form is given as (VI) in the above list. It will be noted that (VI) bears the same relation to (VII) as (II) bears to (III).

Davidson (*5*) has used a growth equation which bears a similar definite relation to Brody's, viz., log $y=a-be^{-kx}$, where y is the growth or weight attained by the animal.[4] The difference between these two equations is interesting. Both are intended to apply to growth only after the *rate* of growth has reached its maximum. After this time, according to Brody's equation, the rate of growth decreases at a rate which is a constant proportion (k) of the rate of growth of the *whole organism.* According to Davidson's equation the rate of growth decreases at a rate which is a constant proportion (k) of the rate of growth of the *average cell* of the organism (assuming the number of cells proportional to the weight of the animal). The k's in the two equations have therefore somewhat different meanings. Brody has applied his growth equation successfully to the age changes in weight of Jersey cows (as well as a great variety of other growth data). Davidson has applied his growth equation successfully to the same data of growth in weight of Jersey cows.

This is an interesting illustration of the often observed fact that the same data may lend themselves to two rather different postulates. If one is to use the conformity of the two equations to the observed values as evidence of the support which they afford to the two postulates, respectively, it is quite evident that some precise method of fitting the equations must be followed. Although it is somewhat apart from the subject of this paper, it has seemed of interest to compare the growth equations of Brody and Davidson when fitted by exact methods. For this purpose the writers have used Davidson's (*5*) Table 7 giving the change in weight with age of original entry Jersey cows. The equations have been fitted by least squares without regard to frequencies. The method will be apparent from the discussion of equation (1). It is necessary to tabulate only values corresponding to those of columns 1, 2, and 4 of Table 2. The fitted equations and the sum of the squared deviations are as follows:

---

[4] This equation was suggested by Wright (*10*), in the form log log $\frac{k}{y}=a(b-x)$.

Equations	$\Sigma\Delta^2$
$y=953.93-277.73e^{-0.512440t}$	745.44
$\log y=2.979189-0.248057e^{-0.547614t}$	748.02

In these equations t is time in years with origin at birth.

As judged by the sum of the squared deviations, Brody's form of expression seems to be slightly better. The deviations are shown graphically in Figure 4. This figure makes it clear that the computed values by the two equations are nearly the same. Davidson's equation conforms to the observed values slightly better at the younger ages, Brody's better at the older ages. But the present method of discrimination does not show any very pronounced choice between the two forms of expression. The question seems to be, shall we say that the rate of decrease in the rate of growth is 51 per cent (Brody) or 55 per cent (Davidson) per annum?

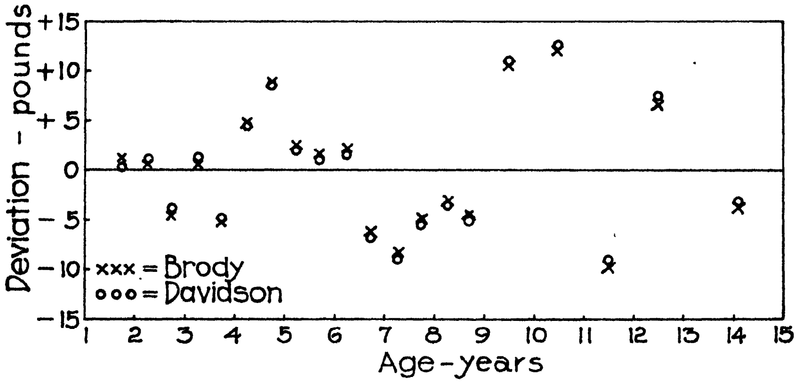

FIG. 4.—Deviation of observed weights of Jersey cows from calculated weights. The same set of observed weights of Jersey original-entry cows has been fitted with the growth equations of Brody (*1*) and Davidson (*5*). The chart shows the observed minus the calculated values

The relation of these growth equations to (VI) and (VII) is readily apparent, and this justifies their consideration in the present connection. Equation (VI) appears to merit more attention as an expression of age changes in the rate of milk secretion. The matter is not pressed further on the basis of the present milk-yield data, however, for the reason that the cows have undoubtedly been steadily weeded out on the basis of their milk yields in previous lactations. Sanders (*14*) has used a method of eliminating, in part at least, the effect of such selection, and the curve resulting from his method is much less asymmetrical than the raw curve such as we are here dealing with. The choice between equations (VI) and (VII) might well rest, it would seem, upon the relative merits of the corresponding growth equations.

SUMMARY

This paper deals with the records of Red Danish cows published in the Danish herdbooks. The records are comparable with American Cow Testing Association records for fiscal years. The curve, due to Davidson, log $y=a-be^{-kx}-ce^{hx}$, has been fitted by the method of least squares to 4,109 annual records of the breed. The calculated maximum rate of production, 9,057 pounds of 4-per-cent milk (3,079 therms) per year is attained during the eighth year of life.

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MORPHOLOGY AND TAXONOMY OF THE PECAN-SCAB FUNGUS, CLADOSPORIUM EFFUSUM (WINT.) COMB. NOV.[1]

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INTRODUCTION

In the southeastern part of the United States the fungus hitherto known as *Fusicladium effusum* Wint. causes a widely prevalent and destructive disease, commonly called scab, on the wild and cultivated pecan (*Hicoria pecan* Brit.). The first recorded collection of the fungus was made by F. S. Earle, who collected affected leaves of the mockernut, *H. alba* (L.) Brit. (*Carya alba* (L.) K. Koch), near Cobden, Ill., October 1, 1882. This collection was sent to G. Winter, Berlin, Germany, who about three years later described (*11*)[3] the fungus as *F. effusum*. In 1888 Langlois collected at St. Martinsville, La., leaves of the pecan parasitized by a fungus that was described and named *F. caryigenum* (*6*). This locality is well within the range of the present known distribution of the pecan-scab disease. Orton (*9*) considered *F. caryigenum* Ell. and Lang. identical with *F. effusum* Wint.

Although the fungus is common on the pecan in the southeastern part of the United States, it has been found only occasionally on other species of Hicoria. During the last three years the writer has made several collections of the fungus on *H. alba* near Thomasville, Ga., and one near Charleston, S. C. Two collections[4] were made at Manhattan, Kans., on *H. cordiformis* (Wang.) Brit. (*C. amara* Nutt.). The fungus was also reported (*3*) on that host from Wisconsin. In 1926 Nolen (*8*) reported collecting the fungus in Florida on *H. aquatica* (Michx. f.) Brit.

THE FUNGUS

The fungus is strongly parasitic and invades only young or growing tissues. It attacks nuts (pl. 1, B), twigs, leaves (pl. 1, A), and catkins of the pecan, but it is known to attack only the leaves of other species of Hicoria. The exact method by which the germ tube enters the host has not been demonstrated. Under favorable con-

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[2] The writer wishes to express to M. B. Waite, Anna E. Jenkins, John W. Roberts, and J. R. Cole, Bureau of Plant Industry, U. S. Department of Agriculture, his appreciation for helpful suggestions and numerous courtesies rendered during the progress of the investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 186.

[4] Specimens in the herbarium of the Office of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture: No. 1897, Ellis and Everhart, North American Fungi, *Fusicladium effusum* Wint., on *Carya amara* Nutt., Manhattan, Kans., September, 1887; and No. 39, Kellerman and Swingle, Kansas Fungi, *Fusicladium effusum* Wint., on *Carya amara*, Manhattan, Kans., June, 1889.

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ditions the incubation period may be as short as 4 to 5 days, but frequently lesions do not become noticeable until a period of 8 to 10 days has elapsed. The disease is rather superficial, extending only slightly below the epidermis. The affected tissues become black, hardened, and somewhat cracked, but show no tendency to decay unless they are later attacked by secondary invaders such as the pink-rot fungus (*Cephalothecium roseum* Cda.). On nut hulls, twigs, petioles, rachises, and leaf veins the fungus forms black stromata, which are raised somewhat above the infected host tissue.

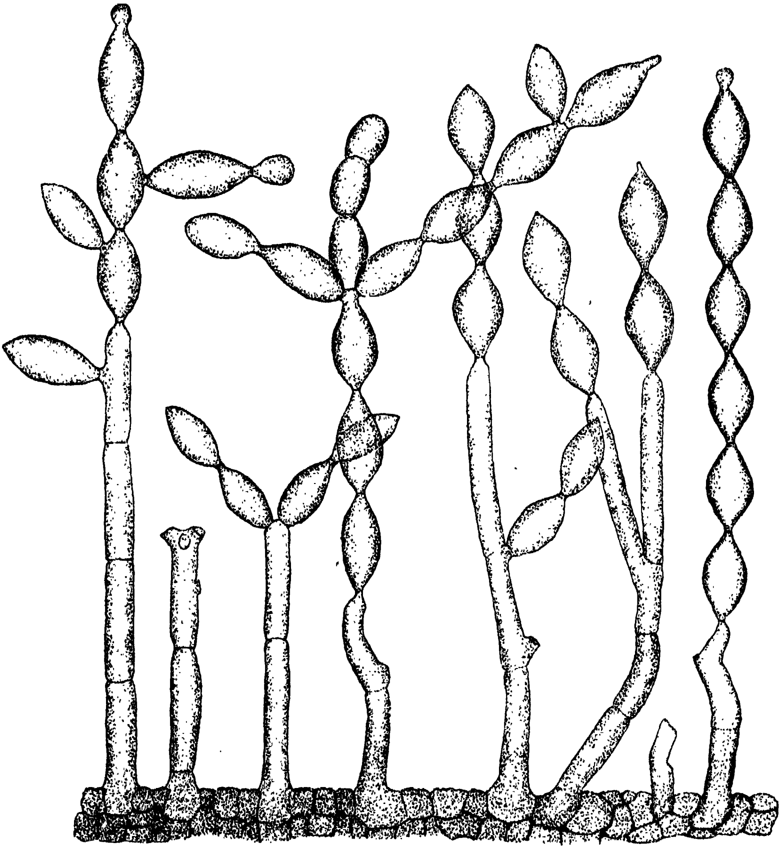
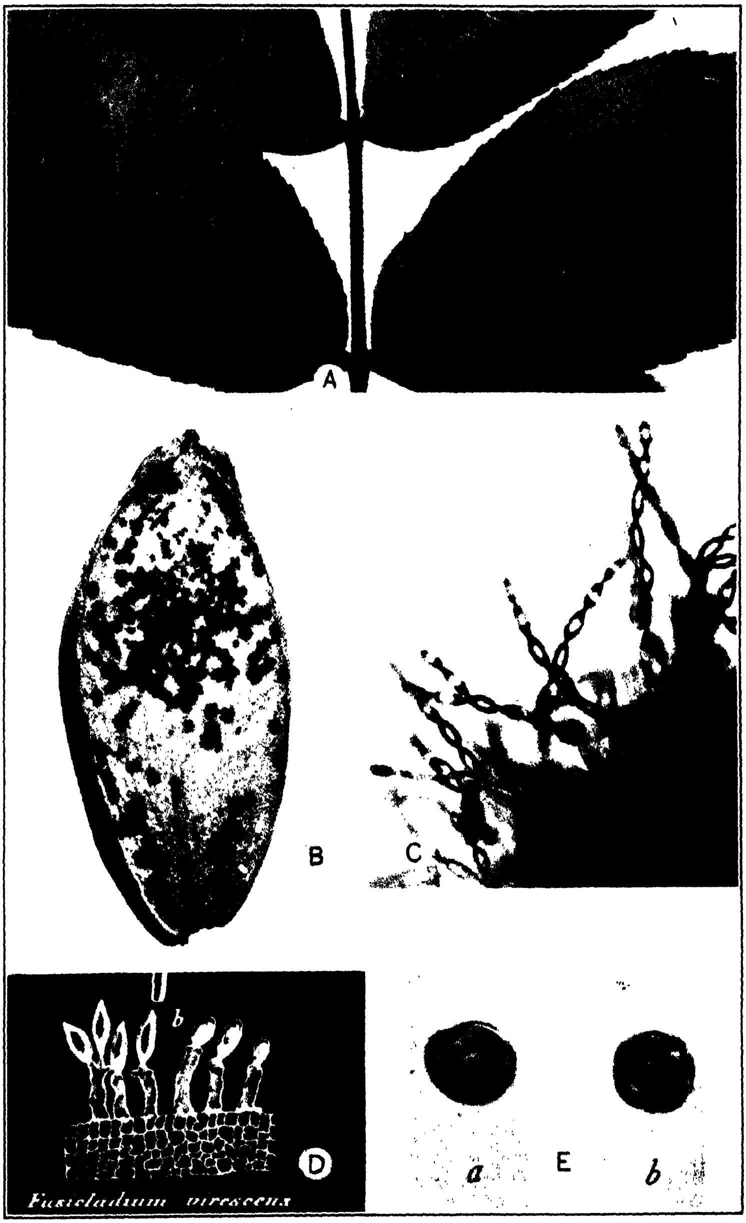


FIG. 1.—Method of sporulation and branching of the pecan-scab fungus. × about 540

The first conidiophores push out through the cuticle, but as the epidermis and the cuticle are destroyed by the anastomosing hyphae the later ones are produced directly upon the surfaces of the newly formed stromata.

The conidiophores are dark brown near the base but lighter toward the tips. They vary in length from 40 to 75 μ and may be either straight or flexuose and either simple or laterally branched. On the host tissues and under natural conditions the conidia regularly and constantly form in chains. (Pl. 1, C, and fig. 1.) The number of conidia in a chain varies from two to nine and probably averages four



A, Pecan leaves typically marked by the pecan-scab fungus. Most of the spots originated on the veins. B, Lesions on an immature pecan nut. The small spots originated from conidia produced on the larger central primary one. C, Chains of conidia of the pecan-scab fungus, photographed upon living host tissue, × 360. D, Reproduction of a drawing by Bonorden illustrating his conception of the method of sporulation of the genus Fusicladium (*1, pl. 4, fig. 94*). E, Parallel cultures of the pecan-scab fungus (*a*), and the peach-scab fungus (*Cladosporium carpophilum* Thüm. *b*), grown on Lima-bean agar for 34 days at a temperature of 24.5° C.

or five. Usually the chains originate at the tips of the conidiophores, but occasionally they develop laterally on the conidiophores at points immediately below septa or less frequently between them. The chains of conidia often branch in an indefinite and irregular manner. The conidia are formed acropetally. The first conidium that is formed on the tip of a sporogenous hypha soon gives rise, usually at its apex, to a papillalike projection. (Fig. 1.) The papilla first lengthens, then assumes a beadlike shape, and finally develops into a mature conidium. In the same way this newly formed conidium gives rise to a third, and in like manner a chain of several successive conidia may be produced. The chains branch by a mature conidium's forming two or more papillae, each of which becomes the starting point of a short chain.

The conidia (fig. 2) are light brown. They vary considerably in both size and shape. In measurements of 200 the dimensions varied from 4.5 to 10 µ in width and from 10 to 28 µ in length. Their average size was 7 by 17 µ. They may be ovate to almost cylindrical, but most of them are either spindle shaped or clavate. All abscised conidia, except those that terminated their respective chains, have both basal and apical scars, showing that they had been attached at both ends. The newly formed conidia are one celled, but some become one or two septate upon germination.

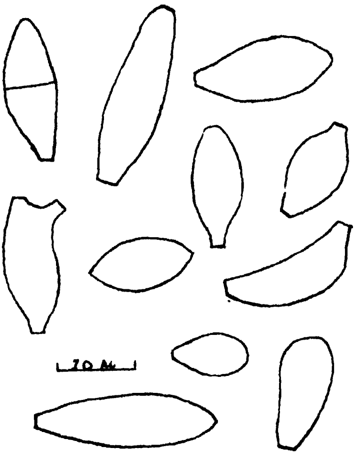


Fig. 2. Conidia of the pecan-scab fungus. Note apical and basal scars on most conidia. (Drawn by the aid of a camera lucida)

METHODS OF DEMONSTRATING THE CATENULATE ARRANGEMENT OF CONIDIA

Conidia of the pecan-scab fungus are so easily pulled apart that the chains are broken up at once when they come in contact with a liquid. Therefore, the catenulate arrangement of the conidia can not be easily demonstrated by the usual method of preparing either temporary or permanent mounts. The breaking up of the conidial chains when coming in contact with a liquid is undoubtedly the principal reason why the true method of conidial production has been overlooked in the past and why the fungus has been classed as a Fusicladium. Conidia mounted in either water or other mounting fluids, no matter how carefully handled, seldom furnish more than mere suggestions that they are formed in chains. In such mounts about the only observable evidences of their arrangement in chains are (1) that an occasional conidium may be found with a bud or immature conidium attached to it and (2) that most conidia show both basal and apical scars.

The following method of studying the manner of conidial formation was found to be very satisfactory: A small section of host tissue, bearing an incipient scab infection, was cut from a living pecan leaf. It was found best to select an infection located on the rachis or some other part largely composed of vascular tissue. The excised host tissue was then sliced very thin with a hand sectioning outfit, and the sections were floated on a drop of water placed on a cover glass. Part of the water was allowed to evaporate, so that the sections would lie flat and adhere closely to the cover glass. The cover glass was then inverted over a culture ring with a drop of water in the bottom, and the joints were sealed with vaseline. Sufficient moisture to favor growth was absorbed by the host tissue and the mycelium. The specimens to be studied were then lying in a moist atmosphere and within focusing distance of a high-power objective. After exposure to a temperature of 24° to 26.5° C. for 8 to 12 hours, chains of conidia were frequently formed from the edges of the sections and in a plane parallel to the cover glass.

The technic just described permitted observation of the progress of sporulation from hour to hour and eliminated the necessity of moving the conidia from their original position during the course of the study. Cultures made by this method will remain for several days in condition for observation. Sections of living overwintered stromata from twigs or hulls will give similar results.

The catenulate arrangement of the conidia can also be demonstrated by carefully forming a crease across a new leaf infection and with the medium-power objective examining the conidia as they project beyond the leaf tissues. To get the best results from this method of observation, new leaves bearing recent infections should be collected and placed in a moist chamber for a period of 12 to 18 hours prior to examination. The chains will then stand out distinctly. Dry mounts will also show the conidia in chains, but in a manner less satisfactory for study. In 1926 the writer (*4*) called attention to the catenulate arrangement of the conidia and pointed out that this character precludes the inclusion of the fungus in the form genus Fusicladium.

The genus Fusicladium was founded by Bonorden with the species *virescens* as the type. (Pl. 1, D.) A translation of his description of the genus is as follows: "Unbranched septate hyphae, bearing at their apices, which sometimes have two projections, one or two spindle-shaped simple spores" (*1, p. 80, pl. 4, fig. 94*).

Engler and Prantl (*7*) list the genus Fusicladium in the Dematiaceae-didymosporae under the subdivision "Conidia not formed in chains." Saccardo (*10, p. 345*) also catalogues the genus Fusicladium in the Dematiaceae-didymosporae and under the subdivision "Conidia not catenulate—merely acrogenous."

COMPARISON WITH THE APPLE-SCAB AND PEACH-SCAB FUNGI

Fusicladium dendriticum (Wal.) Fel., the conidial stage of *Venturia inaequalis*, has probably been studied more closely both in this country and in Europe than any other member of this form genus. In a description of this fungus, Duggar (*5*) describes its method of sporulation as follows:

> These conidiophores arise from the subcuticular or subepidermal mycelium * * * and a spore is soon developed at the tip of each * * *. However, when this spore is abscised, the conidiophore grows further, leaving a slight knee or other evidence indicating the point where the previous spore was borne. In this manner many successive conidia may be produced, and the conidiophore therefore becomes flexuous and irregular.

This description seems to conform quite closely to the general conception of the method by which the species of Fusicladium produce their spores.

Since the resemblance in morphological and cultural characters between the pecan-scab pathogene and the peach-scab fungus (*Cladosporium carpophilum* Thüm.) is much greater than between the former and the apple-scab fungus (*Fusicladium dendriticum*), it seemed to the writer that there might be a closer affinity between the pecan-scab and the peach-scab fungi than between the pecan-scab and the apple-scab fungi. Therefore, as a supplement to this study, comparative studies of the cultural and morphological characters of all three forms were made.

The results of these studies indicate that the pecan-scab organism possesses certain cultural and morphological characters in common with the peach-scab fungus. Both develop in artificial media very slowly at first, monosporous cultures requiring two or three weeks before becoming large enough to be seen macroscopically. Both produce a black stromatoid growth which attains a maximum surface diameter of 10 to 15 mm. in 8 to 10 weeks. Upon artificial media and living host tissues both produce, acropetally, catenulate conidia that are much alike in shape, color, and method of germination. Both produce a superficial growth upon infected host tissues, and both pass the winter as stromata or masses of pseudoparenchymatous tissue. The only essential cultural difference noted was that the pecan-scab organism produces conidia sparsely in culture, whereas young cultures of the peach pathogene produce them in abundance. (Pl. 1, E.)

Many specimens of the conidial stage of the apple-scab fungus on living apple leaves were examined. All methods were employed that had proved successful in studying the conidial production of the pecan-scab fungus, but in no certain case was the apple-scab fungus observed to be producing its conidia in chains. On the other hand, on living host tissues it regularly produces its conidia singly on short mostly one-celled sporophores in accordance with the usual conception of the genus Fusicladium.

These two forms of Fusicladium and Cladosporium were selected for comparison with the pecan fungus on account of their generally accepted validity, accessibility, and adaptibility as types for study. Furthermore, Clinton (*2*) thinks it possible that *F. virescens* Bon., the type species of the genus Fusicladium, may be identical with either the conidial stage of the apple-scab or the pear-scab fungus. The evidence seems to suggest that it is more probably identical with the apple-scab fungus, since Bonorden gives the apple as the host for his fungus.

The results of those comparative studies indicate that the pecan-scab organism possesses certain cultural and morphological characteristics in common with some members of the genus Cladosporium. Its similarity with *C. carpophilum* is so striking that it suggests the probability of close relationship and seems sufficient to warrant its transfer from the form genus Fusicladium to the form genus Cladosporium.

EMENDED DESCRIPTION

The reclassification of the fungus as a member of the form genus Cladosporium is proposed, with, of course, the retention of Winter's specific name. It seems desirable to include in the description of the fungus the hitherto unpublished facts concerning its morphology. The following emended description of the species is therefore given:

Cladosporium effusum, comb. nov. Syn. *Fusicladium effusum* Wint., 1885, Jour. Mycol. 1: 101. *Fusicladium caryigenum* Ell. and Lang., 1888, Jour. Mycol. 4: 124.

Mycelium olive brown, subepidermal at first, later forming pseudoparenchyma of more or less irregular cells on twigs, petioles, and fruits. Stromata formed late in summer remaining in semidormant condition during the winter and sporulating the following spring; conidiophores simple or branched, 1 to 4 septate, dark brown, 40 to 75 μ long. Conidia fusoid to ovate clavate, light olive brown, continuous, occasionally becoming 1 to 2 septate, 4.5 to 10 μ by 10 to 28 μ, catenulate, forming acropetally; chains frequently branched.

Parasitic on nut hulls, twigs, leaves, catkins of the pecan (*Hicoria pecan*), and on leaves of some other species of the genus Hicoria in the central and southeastern parts of the United States.

In artificial culture the fungus grows very slowly. Ten to fifteen days are often required for growth originating from a single conidium to become large enough to be seen macroscopically. When fully developed, which requires from two to three months, the colonies are lens shaped to irregular. The submerged portion is dark brown to black. The exposed part of the stromatoid mass is covered with brown to gray aerial hyphae. Conidia are produced but sparsely in artificial cultures.

The fungus first forms noncolored restricted areas on the host. Later the spots appear black, as the mycelium forms pseudoparenchyma or stromata involving or rupturing the epidermis.

SUMMARY

The pecan-scab organism has for many years been accepted as a species of the form genus Fusicladium. This genus, according to the description by Bonorden, its author, should include only those forms of the Dematiaceae forming one-celled conidia singly or in pairs on short conidiophores.

The result of the present study demonstrates that the pecan-scab fungus forms its conidia in either simple or branched chains. The number of conidia in a chain seems to vary from two to nine, and probably averages four or five.

Simple methods of demonstrating the catenulate arrangement of the conidia are described.

The reclassification of the fungus as a member of the form genus Cladosporium with the name *C. effusum*, comb. nov. is proposed.

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WINTER INJURY OF ALFALFA[1]

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INTRODUCTION

In the spring of 1925 L. E. Melchers showed the writer a serious injury of the crowns and taproots of 1-year-old alfalfa plants grown in Kansas. The injury, which he later described,[3] was characterized chiefly by browning and decay of the outer part of the taproot beneath the crown. (Pl. 1, A and B.) The circumstances accompanying the occurrence of the injury suggested as its probable cause some adverse climatic condition during the winter months. It was found occasionally in several other States in the spring and summer of 1925. In the spring of 1926 similar injury was found by the writer to be widespread and often severe in southern Wisconsin. Here it occurred in plants of all ages and was always present in some of the surviving plants in fields where more or less of the stand had died from winterkilling. In the spring of 1926 J. L. Weimer found in Kansas much root injury, which, upon examination, appeared to be exactly like that in Wisconsin. A detailed description of the outward appearance of these injured plants has been published by Weimer (*7*),[4] who suggested that the origin of the lesions might be winter injury. This term appears well chosen and will be used here inclusively to designate all injuries that appear to be due at least in part to cold during the winter season.

During 1926 Weimer and the writer collected as widely as possible alfalfa plants showing evidences of winter injury in all degrees of intensity, and the writer began an examination of this material to determine as far as possible by histological methods which tissues first show injury and how the later conspicuous lesions develop. Before this work was completed winter injury appeared again in the spring of 1927, not only in southern Wisconsin, where it was discovered and material collected earlier than in 1926, but also in Kansas, Iowa, and other States, where further collections were made and examined. Thus the following description is based on material collected in both 1926 and 1927 in the central Mississippi Valley.

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[1] Received for publication June 1, 1928; issued October, 1928. This study was carried on in cooperation with the Wisconsin Agricultural Experiment Station.

[2] The writer is indebted to many persons for aid in the execution of work presented in part in this paper—especially to Dr. J. L. Weimer, of the Office of Vegetable and Forage Diseases, for many collections of material; to Prof. E. J. Kraus, of the University of Wisconsin, for suggestions in histological studies; to Dr. G. H. Conant for aid in the study of seedling anatomy; and to Dr. Valdimer Skoric for aid in the preparation of the drawings.

[3] MELCHERS, L. E. CROWN AND ROOT ROT OF ALFALFA (UNDET.). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 9:54. 1925. [Mimeographed.]

[4] Reference is made by number (italic) to "Literature cited," p. 211.

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The work has been done not only for the purpose of facilitating the early recognition of the injury in experimental demonstrations of its cause, but also for the purpose of determining its relation to bacterial wilt, which often destroys winter-injured plants.

Before describing in detail winter injury in the crown and taproot of the alfalfa plant, it is necessary to trace the development of the tissues in which the injury occurs.

DEVELOPMENT OF THE ALFALFA PLANT

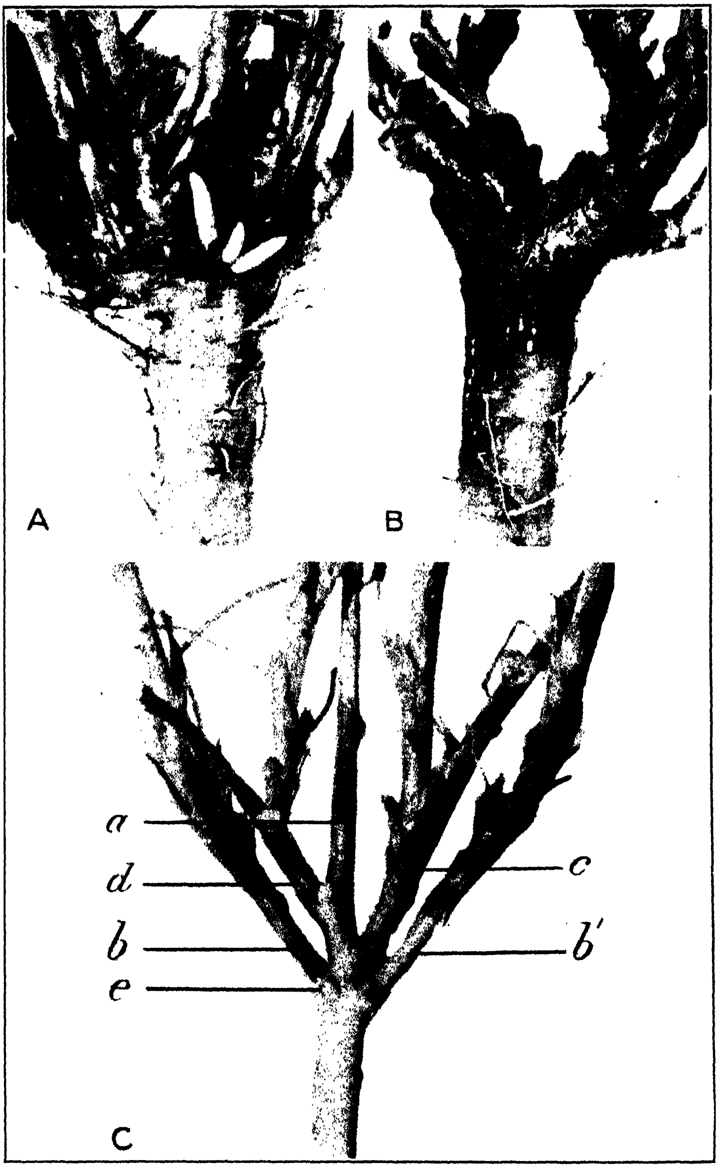
GROWTH CHARACTERISTICS

The general appearance of the alfalfa seedling has been so well described by Compton (*1*) and others that no new description is needed here except in so far as this seedling forms a part of or gives rise to the perennial part of the plant with which we are especially concerned. The seedling root is surmounted by a hypocotyl about 1 cm. long, distinguished from the root by the absence of root hairs. After secondary growth begins and the root hairs on the taproot are lost it is no longer possible to distinguish the hypocotyl readily, and in this paper it will be considered as a part of the upper end of the taproot. The upper end of the hypocotyl is marked by the bases of the cotyledonary leaves, which, together with the first unifoliolate leaf, are the first foliage of the seedling. With the development of the single seedling stem, the first trifoliolate leaf arises above and nearly opposite to the unifoliolate leaf, and other leaves arise in characteristic order.

The branching from the primary axis of the plant, leading to the formation of the characteristic crown, usually proceeds in the following manner: The first stems from the primary axis are usually three in number developing almost simultaneously from the axils of the cotyledonary leaves (pl. 1, C, *b* and *b'*) and of the unifoliolate leaf (pl. 1, C, *c*). In addition to these a stem may develop from the axil of the lowest trifoliolate leaf (pl. 1, C, *d*). The bases of these three or four stems usually constitute the largest branches of the crowns of old plants.

In addition to these stems from axils of leaves, in vigorous plants other stems may arise from buds that are not axillary in origin and form a part of the crown or perennial portion of the stem structure of the plant. The first nonaxillary bud usually arises between the bases of the stems that have arisen from axils of the cotyledons and in a position opposite to that of the first unifoliolate leaf. (Pl. 1, C, *e*.) In fact, if the plant is very vigorous several buds may arise almost simultaneously in this region, and later from any point in the circumference of the plant at or a little above this level. The numerous small stems of which large crowns are so largely composed arise either in an apparently adventitious manner in this region or from the axils of scales and leaves on the bases of stems. The number and habit of growth of these stems vary greatly in different varieties of alfalfa, and within a variety the number is largely dependent on the vigor of individual plants.

As might be expected, young alfalfa plants in thickly seeded fields rarely show the symmetrical development described in the preceding paragraphs. In less vigorous plants some of the first buds are often suppressed and die, leaving the crown one-sided and irregular. If a



A.—Crown and portion of taproot of an uninjured alfalfa plant from a 4-year-old field at Madison, Wis. (October, 1926).

B.—Crown and portion of taproot of a plant similar to that shown in A except that it suffered severe injury in the preceding winter. The blackened condition of the crown and upper part of the taproot and the absence of buds from the base of the crown are evidences of this injury.

C.—Symmetrical crown of a hardy alfalfa plant at the end of its first winter: *a*, The first seedling stem; *b* and *b'*, stems from the axils of cotyledons; *c*, stem from the axil of the unifoliolate leaf; *d*, stem from the axil of the first trifoliolate leaf; *e*, first bud from the base of the crown arising between the stems from the axils of the cotyledons and opposite the stem from the lowest seedling bud.

seedling becomes too deeply buried by soil or even by a mass of foliage close to the ground, the first crown branches may develop from buds higher up on the seedling stem. The position of the crown seems to be determined largely with reference to the soil level. If at any time a plant is buried, buds near the new ground level will form a new crown above the first one; but if soil is washed away from around the plant, buds do not arise from the exposed root to form a new crown at a lower level.

It should be noted that the term "crown" is used here as it has been used by Stewart (*6*) to designate the perennial part of the alfalfa plant derived from stems. It will be shown later that the term thus restricted applies to a region with distinctive histological and morphological characteristics.

ANATOMICAL STRUCTURE OF THE TAPROOT

As a further background for the description of the injuries found in the taproot and the crown of the alfalfa plant, the development of the tissues involved will be briefly traced. The anatomy of the primary structures of the seedling has been studied by Compton (*1*), who was chiefly interested in tracing the transition from the triarch root to the stem structure in the upper part of the hypocotyl. With the details of this transition the present study is not concerned, but it is important to note that in the hypocotyl the xylem groups are separated by parenchyma, which at the upper end sometimes forms a pithlike center of the structure. This central parenchyma is separated from the true pith of the stem by a node at the base of the first unifoliolate leaf where the vascular elements are closely packed at the center of the stem, and the interfascicular cells near the center are elongated axially with pointed ends. The pointed cells usually become lignified, separating the pith of the stem from parenchyma in the center of the root with a woody plate. The extent of lignified tissue at the center of the axis varies greatly. In a Peruvian alfalfa seedling with its crown above the soil the lignification may extend for some distance down into the hypocotyl, but usually it appears to be confined to the node.

With the addition of secondary xylem to the vascular cylinder, the cells of the central parenchyma of the hypocotyl and possibly of the upper part of the root, which can hardly be distinguished from the hypocotyl, begin to enlarge. At first they are round with intercellular spaces, but later they become irregular in shape and more crowded. Parenchyma of the early secondary growth expands like that of the primary structure. Cells contiguous to vessels may divide with walls tangential to the proximate vessel or group of vessels. In vigorous plants more or less expansion of the central parenchyma seems to continue during the first summer of the plant's growth.

In consequence of this expansion of the central parenchyma, the primary vascular strands and the earlier vessels with accompanying fibers of the secondary growth are pushed into more or less sinuous courses, weaving back and forth and anastomosing in the pithlike center of the root. This change in position of the vascular tissue suggests comparison with an apparently similar distortion described by Gravis (*2*) in the vascular tissue of *Crinum capense.* In Crinum the leafy crown of the plant is drawn down into the soil by a conspicuous shortening of the hypocotyl. Gravis found the shortening to be due to the radial expansion and axial shortening of the parenchymatous cells composing the cortex. Now, in fact Rimbach (*4*) has described a marked shortening of that part of the alfalfa root derived from the hypocotyl, which seems from his account to have taken place during the time when this expansion of the central parenchyma was in progress. A comparison of the figures given by Gravis with longitudinal sections of alfalfa roots suggests that if these roots have shortened as described by Rimbach, the mechanism that brought it about may well have been the radial expansion of the central parenchymatous cells, similar to though less in amount than the radial expansion of cortical cells in Crinum. However this may be, the fact that concerns us here is that the upper part of the taproot, especially that derived from the hypocotyl, is distinguished from the rest of he taproot by having at the center abundant parenchyma interspersed between groups of vessels and fibers, which are irregular in course and anastomose freely.

A feature of the primary structure of the hypocotyl that has not been mentioned in the literature is the presence of four bundles of fibers opposite the phloem groups. These fibers are better developed at the upper end of the hypocotyl and usually do not begin to lignify until after secondary growth is laid down, though they are distinguishable by size and position very early. These may be the characteristic pericyclic fibers found in many species of the Leguminosae (*5*).

ANNUAL RINGS OF GROWTH IN THE TAPROOT

In the upper part of the taproot that part of both the wood and the phloem produced by the cambium in the autumn of each year is so different in character from that produced in the summer that annual rings of growth can be discerned similar in character to those in the wood of trees. The autumn growth of wood is distinguished by having vessels of smaller diameter and by the presence of small regular parenchymatous cells with few or no interspersed fibers between the vessels. (Figs. 1 and 2.) These growth rings might be observed by the unaided eye as readily as those in wood if it were not for the fact that the fibers in each year's growth are usually in distinct groups separated by vessels that break the wood into several apparent layers each of which may easily be mistaken for an annual ring.

The annual ring of growth in the phloem is usually as well defined as that in the xylem, though its annual increase in thickness is comparatively small. In the spring and early summer fibers more or less grouped or massed together are laid down opposite each vascular bundle as though to form a new bundle cap. Later a group of sieve tubes and phloem parenchyma with few or no interspersed fibers is laid down beneath the bundle of fibers. When a bundle of fibers is produced in the spring of the following year, the sieve tubes of the preceding year gelatinize and collapse under a pressure that sometimes also crushes interspersed parenchyma. Thus the annual increment in the phloem consists of a bundle of fibers and a group of sieve tubes and parenchyma, which may be somewhat crushed if new fibers have been deposited between it and the cambium. Thus annual rings of growth can often be seen and counted in the phloem of the root as readily as in the wood. Because of these annual rings

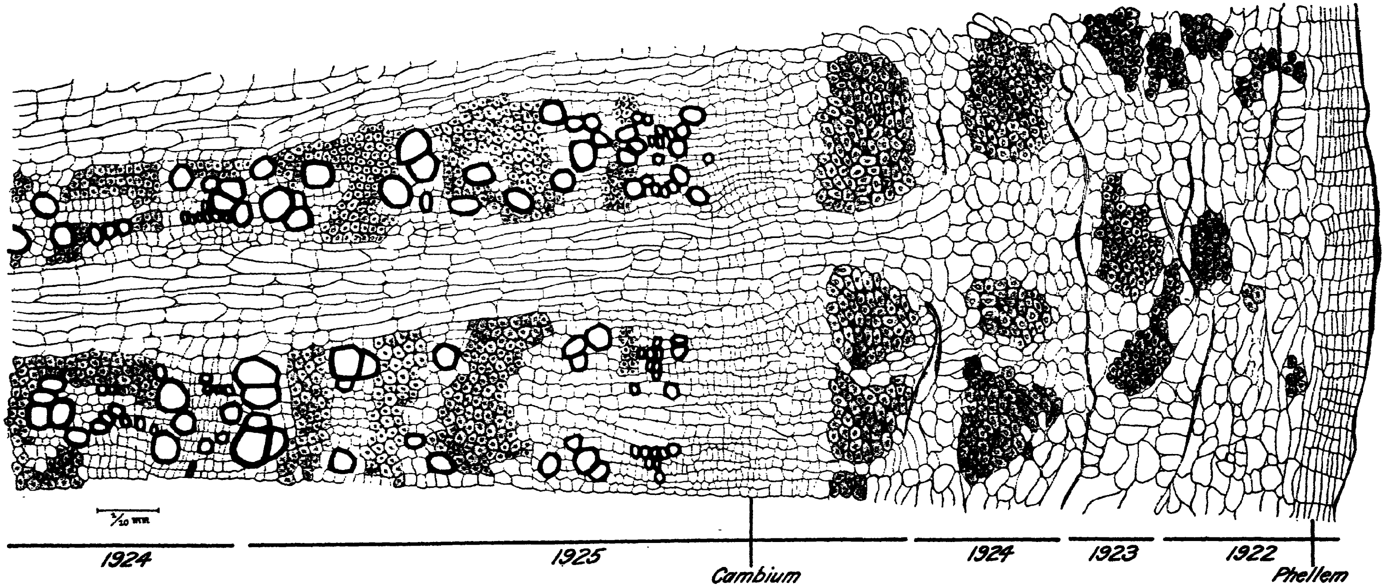


FIG. 1.—Outer portion of a vascular bundle from the upper part of a 4-year-old alfalfa taproot developed from the hypocotyl of the seedling. In this bundle each annual addition in growth is easily distinguished both in the xylem and in the phloem. In the xylem the spring wood has relatively large vessels and abundant fibers. The late autumn wood has few or no fibers and has small vessels surrounded by xylem parenchyma. The end of each annual cycle is marked by the abrupt transition from small to large vessels. In the phloem a bundle of fibers is laid down in the spring, when the xylem is also producing abundant fibers. Sieve tubes and parenchyma are produced in late summer and fall, only to be crushed as soon as the next fibers are produced in the spring. The inner portion of this bundle is shown in Figure 2

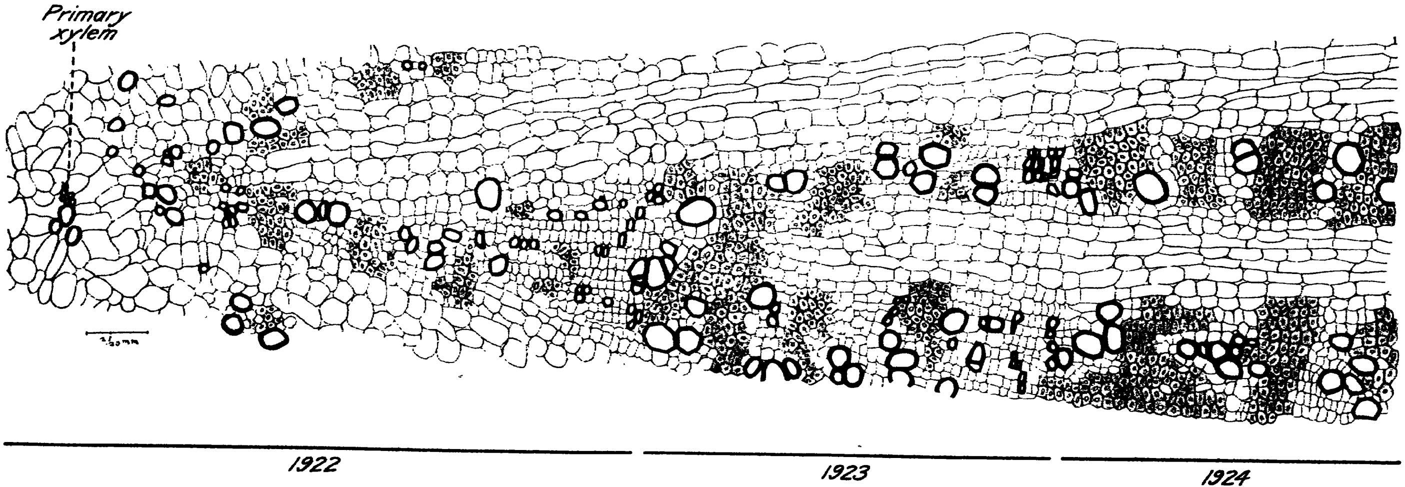


FIG. 2.—Inner portion of the vascular bundle shown in Figure 1. (See legend for Figure 1.)

the year and season in which any portion of the root was laid down by the cambium can usually be determined.

It may be added here that the wood and the phloem of the first year, and sometimes the phloem of the second year, have peculiarities that when recognized facilitate the determination of the age of roots and that are also important in the discussion of the pathological morphology that follows. The wood laid down by the cambium in the first year contains relatively fewer and more scattered fibers than the later growth. The same condition obtains in the phloem where very early in its activity the cambium often lays down a few scattered fibers separated by parenchyma from a later and usually larger group of fibers. When several years of added growth have compressed the early tissue, the fibers of the first year may appear aggregated into two distinct groups. These two groups are usually not completely separated by crushed tissue, or are separated by a zone so narrow and irregular in comparison with the zone of thin-walled tissue in the phloem of subsequent years that no difficulty in determining age need be occasioned by this peculiarity. The same tendency toward the production of two groups of fibers in the phloem of the second year has also been observed, though rarely. It has not been seen in the phloem of later years.

It should be noted here that alfalfa roots from certain regions offer great or even insuperable difficulties in age determination. In roots over 10 years of age from the dry land of western Kansas the annual increment in diameter is often small and uniform in character. Collections from southern California, where the plants do not become dormant in the winter, also fail to show easily distinguishable bands of autumn growth. With these exceptions, however, among 40 collections of known age in the Mississippi Valley from Alabama to Minnesota only 1 collection failed to show internal evidence of age corresponding to the known age of the plants. In this 1 exceptional collection from Manhattan, Kans., two distinct annual rings were deposited in the second year.

ANATOMICAL STRUCTURE OF THE CROWN

The alfalfa crown is here described as that part of the stem structure that is perennial. The ability of the crown to withstand the winter may be due in part to its protected position, but it also seems to be associated with certain peculiarities in secondary growth that should be noted. Even in its primary structure the stem base suggests in some characteristics a transition between root and stem. At the base there is usually a well-defined endodermis (fig. 3), which is less easily distinguished and finally disappears at higher levels. The fascicular and interfascicular cells become heavily lignified, forming a cylinder of strong supporting tissue.

The cambium is very active in the stem bases, especially in the first axillary stems, which will form the large crown branches. When the cambium begins to produce secondary growth the endodermis becomes a phellogen. The portions of the endodermal cell walls inside the thickenings, if any have been laid down, expand conspicuously, and almost simultaneously two or three cross walls are laid down interior to these thickenings. Hereupon the epidermis dies, separates from the cortex, and forms the brown membranous covering often seen on young stem bases early in spring. The phellem

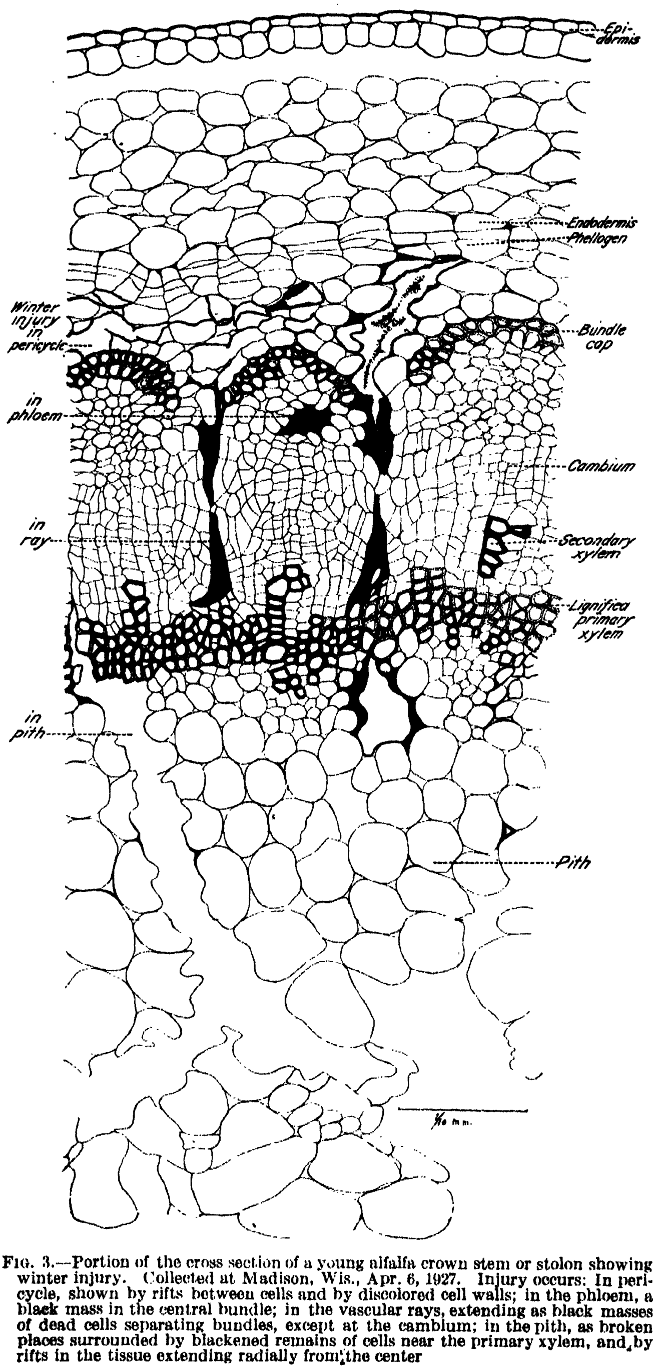


FIG. 3.—Portion of the cross section of a young alfalfa crown stem or stolon showing winter injury. Collected at Madison, Wis., Apr. 6, 1927. Injury occurs: In pericycle, shown by rifts between cells and by discolored cell walls; in the phloem, a black mass in the central bundle; in the vascular rays, extending as black masses of dead cells separating bundles, except at the cambium; in the pith, as broken places surrounded by blackened remains of cells near the primary xylem, and by rifts in the tissue extending radially from the center

originating from the endodermis becomes the effective outer covering, both in the large crown branches from axillary buds and in the small stems. The cambium, which in the beginning may have made some addition to the heavily lignified central cylinder of the stem, begins sooner or later to lay down tissue that is indistinguishable from that which is being produced in the root. The lignified interfascicular cells give way to wood rays, which may store starch, and the structure appears to function in all ways like a root except that it seems able to give rise to buds. The manner in which these buds arise has not been studied. The transition from stem to root structure may occur suddenly, or gradually, or irregularly about the stem. After the change from stem to root structure has been made, the cambium rarely reverses the transition and produces stem structure again, though occasionally small islands of lignified tissue occur in the root structure. Thus occasionally large crown branches buried in the soil for several years become so rootlike in character that their origin is revealed only by the presence of pith and the lignified stem structure at the center.

METHOD OF STUDY

COLLECTION OF MATERIAL

A large part of the plants examined in the beginning of this study was collected in the course of an investigation of bacterial wilt with which some of them were infected. When in the spring of 1926 lesions that are here interpreted as winter injury began to become visible in wilt-infected plants, it was not always possible to distinguish with certainty some of the discolorations caused by winter injury from those caused by the parasitic bacteria. It was soon evident that a thorough knowledge of the character of winter-injury lesions must be gained before the two pathological conditions could be always distinguished. In the last days of April, 1926, the collection of plants showing what was regarded as winter injury was begun. At this time plants were beginning to grow and lesions were not only discolored and easily visible but the injured tissue when sectioned stained in a characteristic manner by the method described below. From this time onward the development of the injuries and the growth response of injured plants were followed through the growing season.

In 1927 it was possible to follow the development of lesions from the time frost left the ground through the spring. Fortunately for this work, heavy ice sheets formed in late winter in some alfalfa fields in southern Wisconsin, killing a large part of the stand. When the ice began to melt, plants that had been buried in ice so long that their death or injury was anticipated were chopped from the frozen ground and examined. In this way winterkilling and winter injury were traced from the time the plants thawed until early summer.

In the following pages the characteristic location of injury of different degrees of intensity and in plants of different ages will be traced. Finally, the evidences of injury as they may be seen with the aid of razor sections will be traced from the beginning to the appearance of characteristic discolored lesions.

PREPARATION OF MICROSCOPIC SECTIONS

From the very beginning of this work it was necessary to make and stain microscopic sections from many plants. A method suggested by E. J. Kraus gave the most satisfactory results. The segments of roots were embedded in parowax in the usual manner. If the material could not be sectioned at once without tearing the sections, the cut ends of the embedded roots were placed in water until they were sufficiently softened to cut easily. A soaking varying from 6 to 24 hours was usually enough. A well-sharpened microtome knife of excellent quality was used, but even with this it was not often possible to cut more than a dozen sections at one place on the blade before the edge was dull and the sections began to tear. With proper care, however, it was possible to get such perfect sections of almost any root that any mechanical injuries produced in roots by freezing were not confused with disarrangement of tissue in the preparation of the sections.

It is fortunate indeed that the method used in staining the parasitic bacteria served to differentiate injured tissue. The method of differential staining used was as follows: The segments of roots were usually fixed in formal-acetic alcohol. The sections were first stained in dilute safranine long enough to give a red color to vessel walls but not to walls of parenchymatous cells. Overstaining in safranine prevented the desired action of the stains that followed it. After standing in water a few minutes, the sections were stained according to Gram's method as described in manuals of bacteriology, except that each slide was dipped in water after immersion in the iodine solution to prevent the appearance of iodine crystals after dehydration in alcohol. It should be noted that the gentian violet and the iodine solutions as usually prepared for this method are at least three times as concentrated as necessary, and that dilutions can be used with equal success, and even to great advantage if the sections contain much starch, which will be blackened by the strong iodine. Diluted stains do not keep and are useless after a few days. Instead of diluting the aniline gentian violet and iodine it is often possible to reduce the time in them to 10 seconds with satisfactory results. Following immersion in the iodine solution, the slides were dipped in absolute alcohol until the stain no longer appeared to run from the sections when the slide was lifted. They were then flooded with a saturated solution of Orange G in clove oil to which about 20 per cent of absolute alcohol had been added. This solution stained the walls of the parenchyma a deep yellow and also removed surplus gentian violet left by the alcohol. If the gentian violet was not removed sufficiently by the clove oil in one or two minutes, the slide was returned to the absolute alcohol for a few seconds for further clearing, and again flooded by clove oil. It was then cleared in xylol and mounted in the usual manner. In sections treated in this way the parasitic bacteria (*Aplanobacter* [*Phytomonas*] *insidiosum* L. McC.) stained a deep blue, while the walls of parenchyma that had been winter injured appeared black, slate gray, or even red, depending on the time that had elapsed since freezing, as will be explained later.

This method appears to be adequate for differentiating the pathological conditions resulting from winter injury from those resulting from bacterial wilt, even when both occur together in the same part of the plant. It has been used exclusively in the preparation of sections from which drawings have been made.

MICROSCOPIC APPEARANCE OF INJURED TISSUE IN ROOTS AND CROWNS

In microscopic sections of injured plants the presence of injury is disclosed by the disarrangement of cells and by the way in which they stain. The position of the injury is definitely characteristic, as will be shown later. Two types of cells, parenchyma and vessels, are chiefly involved.

INJURY IN PARENCHYMATOUS TISSUE

From an examination of Figures 1 and 2 it is readily seen that the pithlike cells at the center of the root and the wood and phloem rays together with abundant phloem parenchyma form a continuous mass of similar cells broken only by the cambium, which is not always visibly different from the tissue that it has produced. In this cell mass is found the larger part of the injury that gives rise to the visible lesions on the plant.

In plants injured during their first winter the cells that show injury first are usually located in the phloem and the phloem rays. The injury may occur wholly in the large cells exterior to the bundle or groups of fibers. (Fig. 4.) Oftentimes the dead cells form a sheath surrounding the root, whereupon all cells exterior to it die and slough off, after which the root has a roughened surface. Injury in the phloem is usually accompanied by more or less necrosis of the large cells at the center of the root and also by lesser injury in the wood rays. (Fig. 5.) Plants injured during their first winter may survive even when little root tissue besides the cambium and the outer portion of the vascular bundles (fig. 6) is left alive below the crown.

In plants injured during their second or any subsequent year the injury usually appears first in the parenchymatous cells of the phloem and phloem rays, as in 1-year-old plants, but the characteristic position of the dead cells is somewhat different, being inside rather than outside the last group of fibers in the phloem (fig. 7); and while the necrotic ring may cross the phloem rays, as described previously, it usually follows inward through the cambium along the wood rays to a depth not exceeding the last annual increment in growth. Thus the cambium is broken at frequent intervals, but the injury does not extend all the way to the center of the root so frequently as in younger plants. The pithlike center is injured as in younger plants, though perhaps not quite so abundantly with increasing age. Accompanying the injury already described and sometimes independent of it, there may be a narrow more or less continuous zone of necrotic cells beneath the phellogen close to the characteristic layer of cells containing crystals. Since the inner walls of the cells containing crystals stain much like those of injured cells, it is sometimes difficult to distinguish true injury in this region. (Fig. 8.)

In the crown stems injury is altogether similar to that in the roots. (Fig. 3.) The large cells beneath the phellogen derived from the endodermis and the large central pith show injury first. The ray cells are easily destroyed and the bundles become separated by dead cells as in the root, though they may be held firmly together mechanically by the inner band of primary xylem. Phloem parenchyma may be injured as in the root. When the crown has begun to be rootlike in character it suffers injury like that of roots.

The precise nature of the action of frost upon the alfalfa root, which gives rise to the injuries described here, can not be determined merely from observation of the injury. It appears, however, that the injury is always found where cells have been separated by rifts along the middle lamellae. These rifts when extensive may result in the physiological isolation and death of tissue. Usually, however, it appears that the rifts are only incidental to injury of a very different

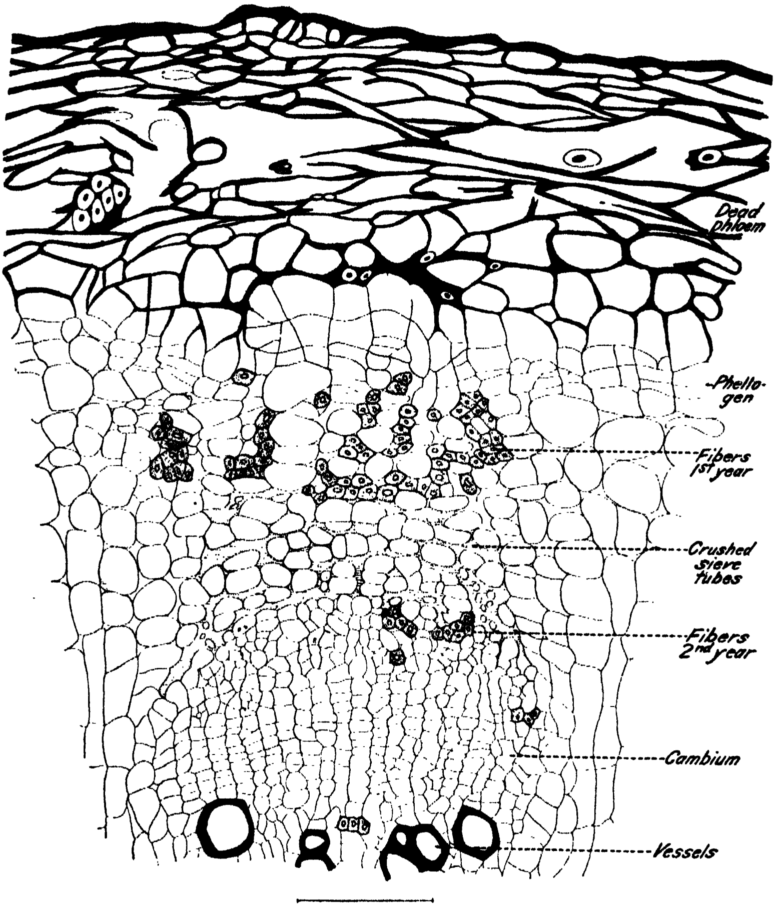


FIG. 4.—Cross section of the outer portion of one vascular bundle of an alfalfa root showing characteristic injury inflicted during the first winter. Grimm alfalfa planted in Doniphan County, Kans., in 1925, and collected Apr. 17, 1926, after it had begun vigorous spring growth that had crushed the phloem produced in 1925. The injury occurred in the phloem exterior to the group of fibers, not only in the bundle, as shown here, but also in the phloem rays, resulting in a ring of dead cells all around the outside of the root. Cells exterior to the injured ring died and collapsed. Meristematic activity of cells below the injured ring was forming a new phellogen layer that would protect the root when the dead tissue decayed

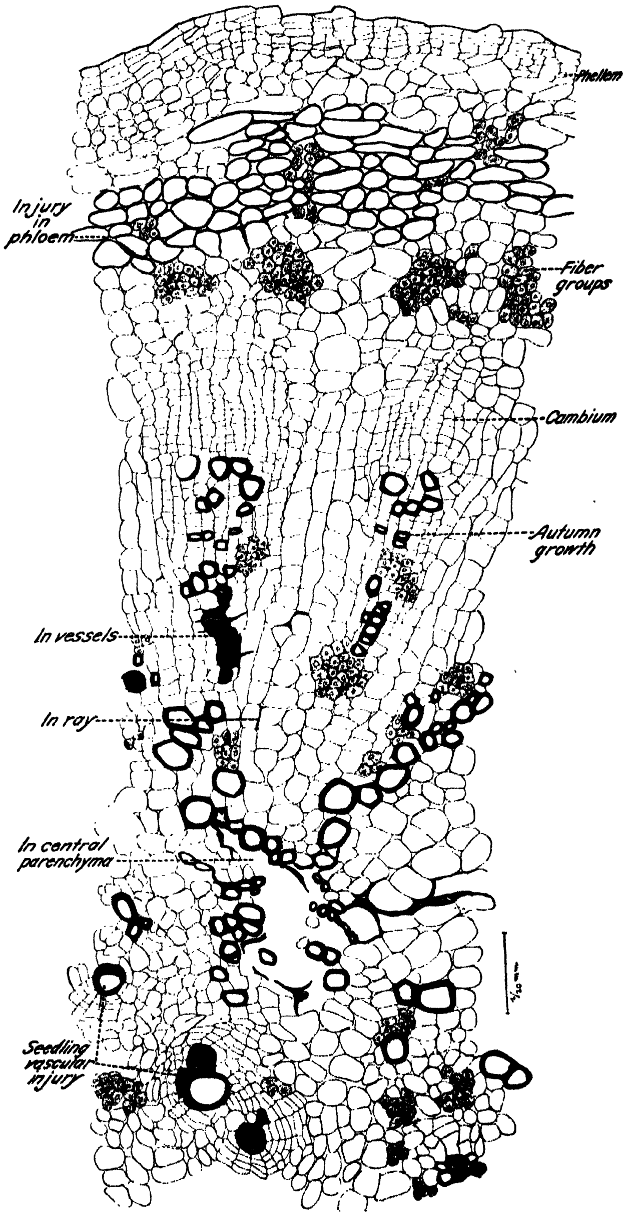


FIG. 5.—Sector of the cross section of a small alfalfa root of a nonhardy variety showing injury incurred during its first winter. Planted Aug., 1925, at Madison, Wis.; collected May 14, 1926, after growth had begun at the cambium. Injury occurred in the phloem like that shown in Figure 4, but no meristematic growth of the cells beneath the injury had begun in this case. Winter injury is shown also in the vascular bundles, in the wood rays, and in the central parenchymatous tissue. The vessels at the center of the root around which abundant cell division had taken place were injured during the previous summer. Such seedling injury is very common

character from the wounding of the tissue that these rifts occasion. This injury expresses itself by a deposition of material in and outside of cell walls. This material gives to these cells the staining characteristics previously described. The cells thus injured do not divide in response to the injury, but when located near the exterior of the plant are usually cut off eventually from the uninjured tissue by the deposition of suberin in the walls of cells located between them and the cambium. From this behavior the writer infers that the injury is primarily a direct effect of freezing, and not primarily or necessarily a consequence of the mechanical separation of cells, which appears also to be caused by the cold.

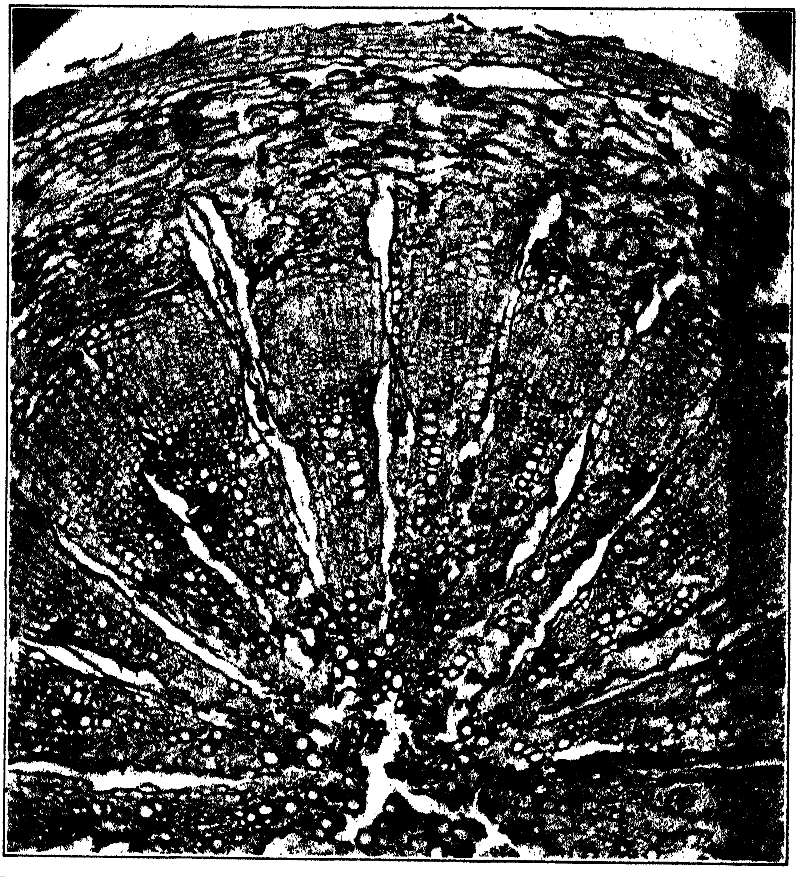


FIG. 6.—Photomicrograph of a portion of a section of the upper part of the taproot of an alfalfa seedling that had suffered severe winter injury during its first winter. Collected at Madison, Wis., Mar. 30, 1927. All tissue except fascicular cambium and adjacent immature cells appear to have been killed. The groups of living cells in the bundles had begun to expand and mature to furnish vascular connection to the buds above. The dark-staining material characteristic of winter injury occurs more or less abundantly at the margins of open spaces bordered by living or injured cells

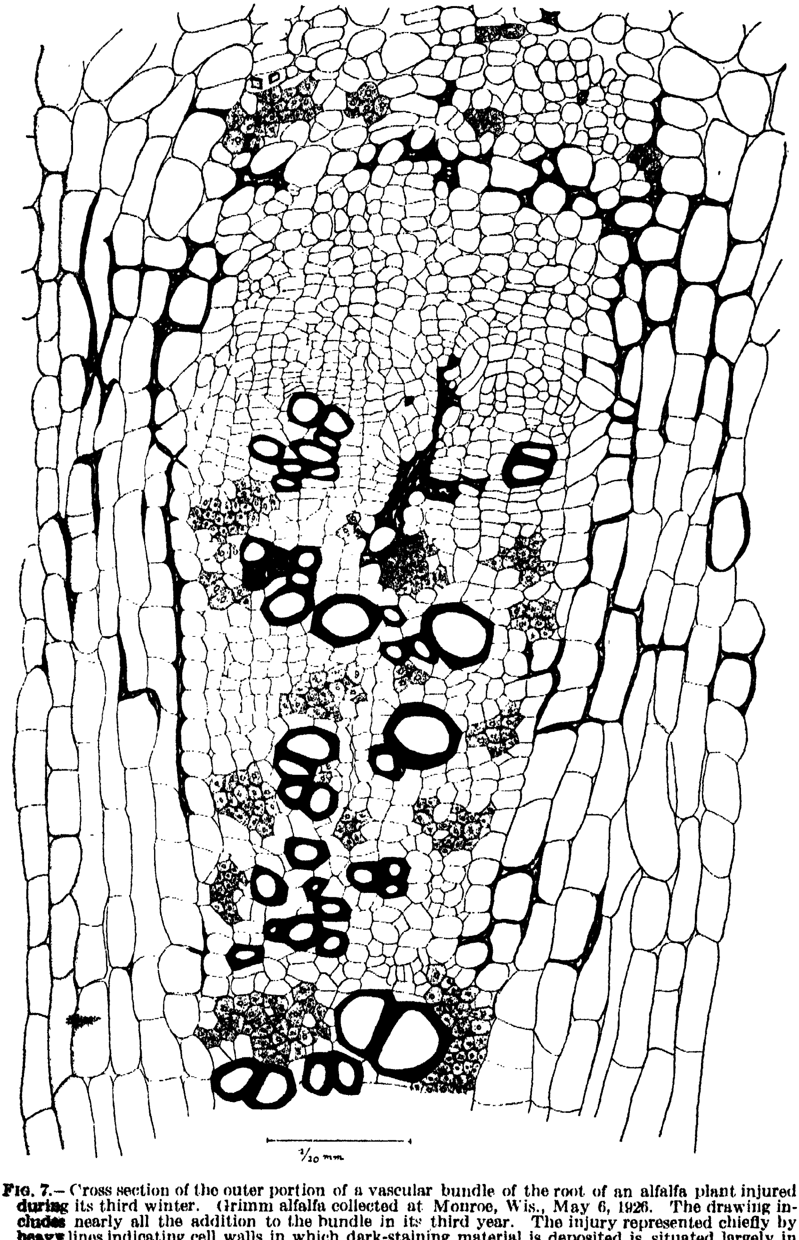


FIG. 7.—Cross section of the outer portion of a vascular bundle of the root of an alfalfa plant injured during its third winter. Grimm alfalfa collected at Monroe, Wis., May 6, 1926. The drawing includes nearly all the addition to the bundle in its third year. The injury represented chiefly by heavy lines indicating cell walls in which dark-staining material is deposited is situated largely in the parenchymatous cells of the phloem inside the innermost group of fibers and in the phloem and the wood rays. Injury in the vascular bundle is shown by a group of disorganized parenchymatous cells in the autumn growth and by two plugged vessels near the disorganized cells. This drawing should be compared with Figure 4, which represents a similar characteristic injury in a 1-year-old plant, and with Figure 9, which represents a less usual type of injury in a 3-year-old plant

INJURY IN THE VASCULAR BUNDLES

Injury in the vascular bundles is limited almost exclusively to cells produced during the previous growing season. It is first discerned in early-spring collections by the presence, in the lumina of vessels, of granules that stain like the walls of necrotic cells. Frequently one or more contiguous parenchymatous cells stain like injured cells in the phloem. If fibers are close to injured parenchyma, they are usually shrunken and stain deeply with safranine. Later in the summer injured vessels become plugged with a gumlike material (fig. 9), unless perchance they are in the center of a necrotic area. Tyloses sometimes appear, but they are always very small, never reaching a diameter equal to one-half that of the lumen of the vessel. Tyloses have been observed only in vessels of injured tissue. The distribution of plugged vessels in the outermost ring of growth shows great variation in different plants. Sometimes plugging is found chiefly in the largest vessels, sometimes chiefly in vessels of autumn growth, and at others in scattered vessels of different size and age. In nearly all the collections from Wisconsin, but not in those from Kansas, whenever there is injury in the last annual ring, a few small vessels of autumn growth in each ring previous to the last have a staining reaction like injured parenchymatous cells and may also be shrunken and separated from adjoining cells.

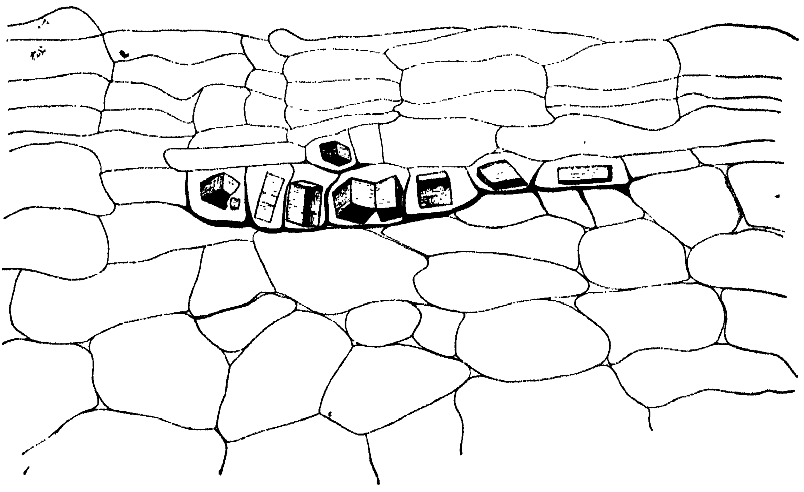


FIG. 8.—Crystals in cells situated beneath the phellem. The inner walls of these crystal-containing cells are much thickened and often stain like the walls of injured cells. Thus they are easily mistaken for injured cells, especially since the crystals are not often conspicuous

In 1-year-old plants injury in vessels is associated almost always in varying amount with injury in parenchyma. In older plants it is on the whole less abundant and severe.

The origin and the composition of the material with which vessels become plugged have not been examined. In appearance the material resembles that formed in vessels of plants infected with bacterial wilt. Similar material has been found in vessels of plants into which

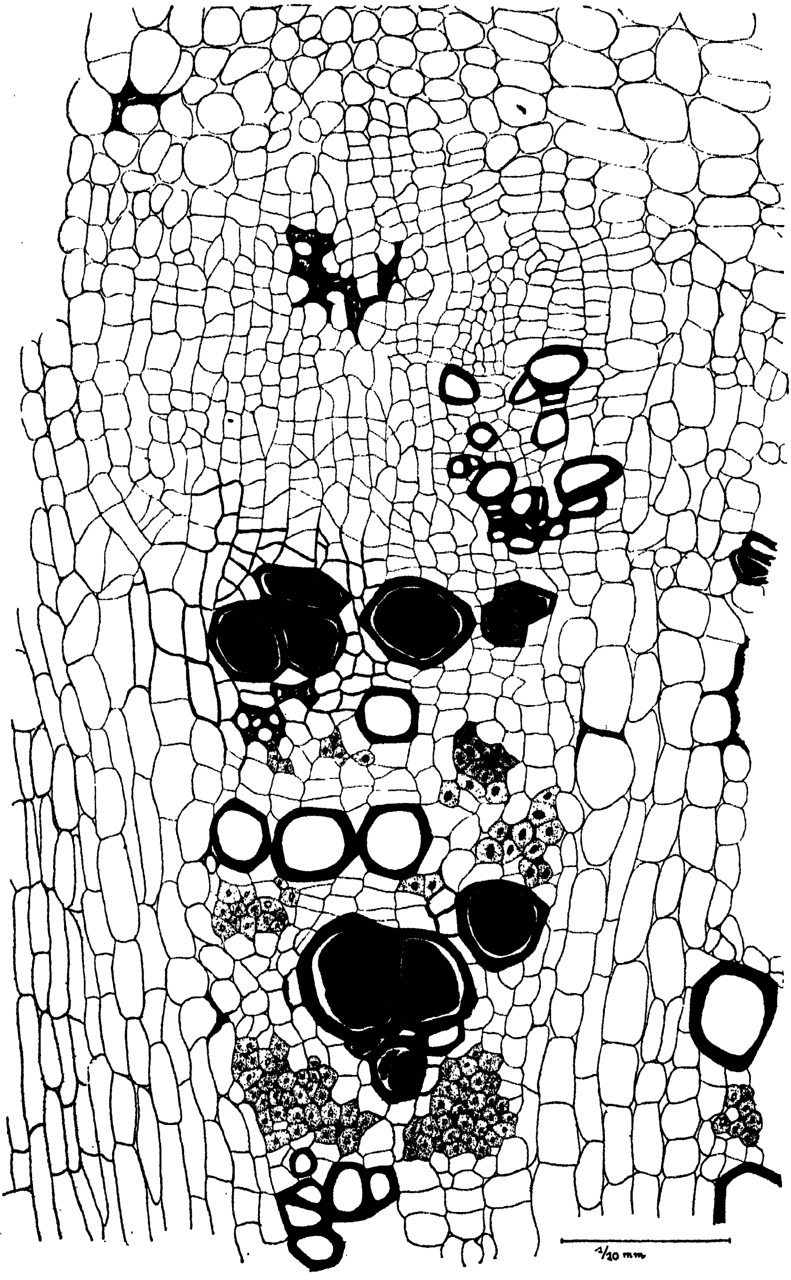


FIG. 9.—Cross section of the outer part of a vascular bundle of the root of an alfalfa plant injured during its third winter. Grimm alfalfa collected at Monroe, Wis., May 6, 1926. The drawing includes all of the addition to the bundle during its third year. This root and that shown in Figure 7 were collected from the same field. The injury represented here is less common than that illustrated in Figure 7. It occurs almost wholly in the bundle, and is shown by the plugging of vessels and the deep-staining walls of some of the adjoining parenchymatous cells represented by heavy lines. A small group of dead cells occurs near the center of the cambium of the bundle as in Figure 7, with which this drawing should be compared

dilute acids have been introduced through cut stems. Presumably the material is the product of living cells contiguous to vessels, and since in some cases it is evident that some of these have been injured, it is not unlikely that some product from them is the inciting cause of the deposition.

DEVELOPMENT OF WINTER INJURY IN RELATION TO WINTERKILLING

Now that the character and position of winter injury in surviving plants have been described, it is of interest to trace the development of evidence of winter injury in several Wisconsin fields in the spring of 1927. On February 24 one collection was made from a 4-year-old field of Grimm alfalfa on which large ice sheets had remained for weeks. At that time the last of the largest ice areas was melting, and inasmuch as no severe freeze followed during the spring, it is assumed that all or nearly all of the damage found later had been done previous to that date. A number of plants were cut from frozen ground beneath the ice where winterkilling or severe injury was anticipated. When the plants were thawed the upper parts of the taproots looked more or less water-soaked, but none of them was conspicuously softened. These roots were embedded and sectioned. Sections of some of the plants showed tissue almost completely disorganized; rifts extended the entire length of the rays, the larger groups of parenchymatous cells had separated into loose aggregations, and the cells of the cambium region were collapsed, though coherent. In some plants nearly all of the tissue except the cambium was disorganized, while in others rifts were found only along the rays. No unusual staining reaction of injured cells was found.

On March 9, when the frost was out of the ground on southern slopes, most of the dead roots in the field previously mentioned were so much softened that they could be recognized. Many roots were much water-soaked in appearance, though fairly firm. Such roots when sectioned showed disorganization of tissue of greater or less extent although most of the cambium was apparently turgid and living. When turgid cells were situated along the margins of rifts they often gave a faint staining reaction characteristic of winter injury. The dead and injured tissue did not at this time show visible discoloration by which it could be recognized in the field.

On March 30, roots of alfalfa at Madison had begun to grow, and injured tissue in roots was usually so much discolored that it could be seen from examination of plants in the field. The condition of the root of a small seedling that barely survived its first winter is shown in Figure 6. Here only small islands of cells with the fascicular cambium at their center had survived, and new conducting elements to support the living buds above were beginning to mature. The islands of living cells were more or less surrounded with the material that takes the characteristic winter-injury stain.

By the end of April all the dead and injured tissue was visibly discolored, and sometimes a stain diffused from it into the uninjured tissue. In one field taproots completely black, almost like ink when cut across below the crown lost most of this color if soaked in water, and when sectioned showed a vigorous living cambium. After the plants had begun to grow vigorously the precise limits of injury were easily discovered by cutting the root across with a knife, though several months might elapse before the outer phloem decayed sufficiently to produce the roughened appearance shown in Plate 1, B.

The characteristic appearance of cross sections of injured roots after growth has started vigorously is shown in Plate 2. In Plate 2, A, the injury is largely confined to the phloem, which is beginning to blacken. Black dots in the wood indicate groups of vessels that are showing the presence of injury by gum deposit and discoloration of a few surrounding parenchymatous cells. A greater degree of injury is shown in Plate 2, B. The death of tissue at the center of the root and along the wood rays had led to extensive cracking of the wood and opening of the center from the pressure of the vigorous new growth. Although the limits of the new growth can be seen clearly almost all the way around the periphery, a stain from the decaying tissue obscures the healthy tissue in one small sector. In spite of this injury, the growth of this plant seemed scarcely less vigorous than that of uninjured plants. At this stage the exact limits of the injury are best seen in stained microscopic sections. At all the small isolated places where cells seem to have been separated, the characteristic staining material has been deposited on the cell walls along the rift. So long as the plant lives it retains these evidences of injury—evidences that will usually reveal upon examination the year in which they occurred. Thus it is possible by the examination of a number of plants to discover the years in which any old field suffered especially severe injury. An instance in point may be cited. One of the disastrous winters for alfalfa in southern Wisconsin was that of 1921–22. Among the collections of wilt-infected plants from the southern part of the State made in 1925 and 1926 were two from fields that were sown in 1921 and that had therefore survived this winter. An examination of sections from these collections showed that almost every plant had in its first year's wood plugged vessels characteristic of winter injury as well as phloem injury when the phloem was present. It may be added here that the examination of other collections of wilt-infected plants from Kansas showed that while there was extensive winter injury in 1924–25 and 1925–26 in eastern Kansas, little of such injury originated in the two preceding years.

GROWTH RESPONSE OF INJURED PLANTS

The growth response of plants to climatic injury appears to be similar to their response to mechanical wounds. All of the parenchymatous tissue in which the larger part of the injury takes place seems to be able in varying degree to become a meristem capable of laying down a cork layer except perhaps the wood ray cells that have become considerably elongated axially. Injury in the phloem and in the central pithlike parenchyma meets the quickest response in the surrounding cells. In the phloem parenchymatous cells beneath those injured quickly begin to divide (fig. 4) and a new corky covering is produced like the original phelloderm. If the new cork arises in immature cells near the cambium it is usually unbroken and effectual. If it arises in mature phloem parenchyma it is usually interrupted by groups of fibers, which pursue a sinuous course through this tissue, and through these fibers decay traverses the cork layer into the underlying cells. These cells usually are able to divide rapidly enough to prevent the decay from progressing far, but in so doing the outer bark is sometimes ruptured by the new growth beneath and new repairs must be made until the outer appearance of the root becomes very rough and irregular.

Healing in the central pith takes place in a similar manner. Small wounds in the first year's wood are usually healed quickly and effectually (fig. 10) if no fibers are involved. Groups of fibers cause difficulty here as in the phloem.

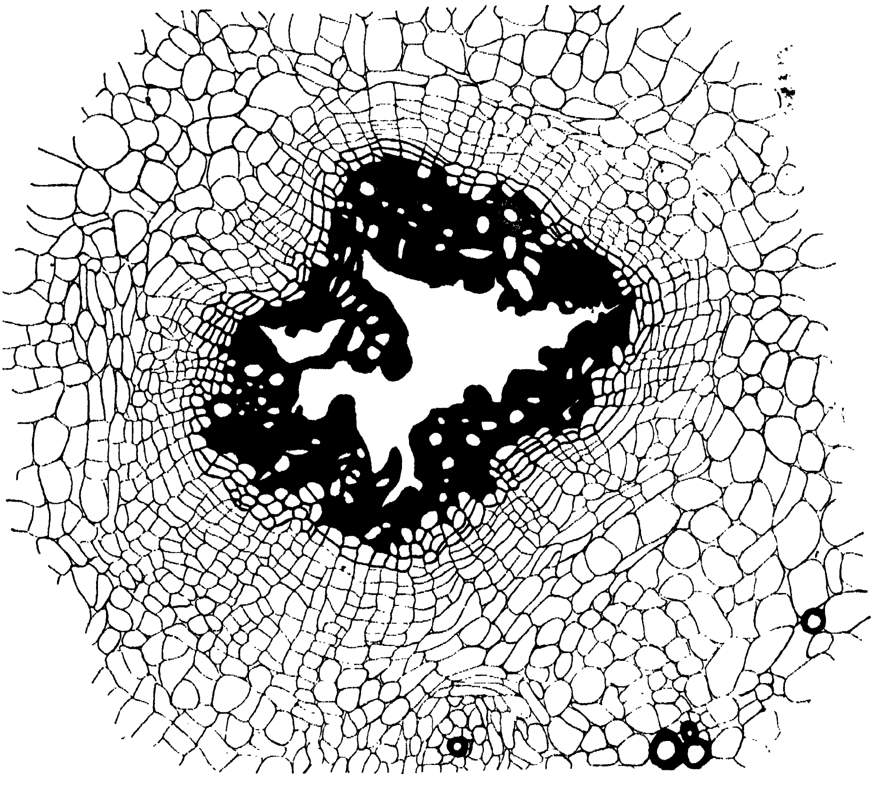
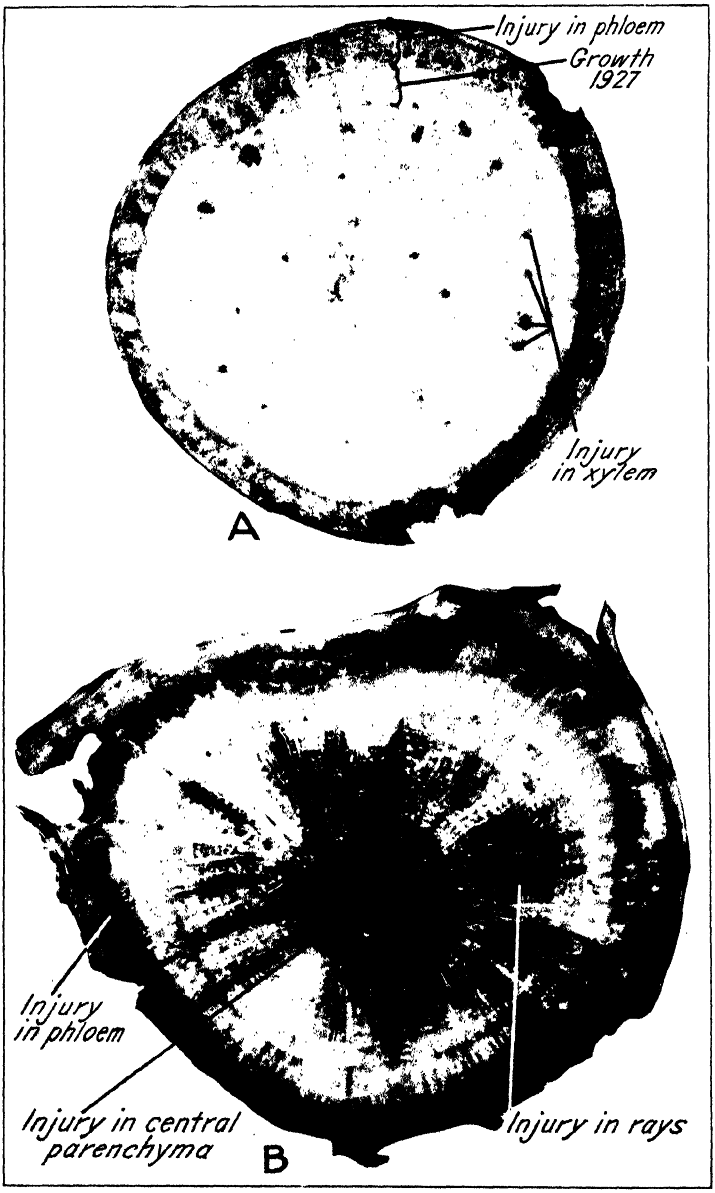


FIG. 10.—Small discolored spot at the center of a 6-year-old alfalfa plant of a hardy variety at Madison, Wis. This spot may have originated from injury in the central parenchyma during the first winter. It has been so well surrounded by the meristematic activity of parenchyma that further decay is prevented

Repair of injury along the wood rays that are completely split open takes place by division of smaller cells along the margin of the bundle which belong perhaps to the wood parenchyma. These cells are not very active in division, especially when they are a year or more old, and if they do divide extensively the mechanical push in a tangential direction that results may break the interfascicular cambium if it was previously intact at this point, or in any case may cause an increase in diameter of the root or swelling that is characteristic of badly damaged plants that have grown rapidly afterwards. Thus the swollen roots consist of a ring of isolated vascular bundles. In Figure 11 is shown a section of one of the segments of such a ring,



Taproots of alfalfa plants showing winter injury, collected in Guthrie County, Iowa, May 17, 1927, after considerable spring growth had been laid down; injury occurred in the second winter that the plant passed through; cross sections of upper parts:

A.—Slight injury occurring chiefly in the phloem.

B.—Severe injury occurring in the phloem, the central parenchyma, the rays, and the wood.

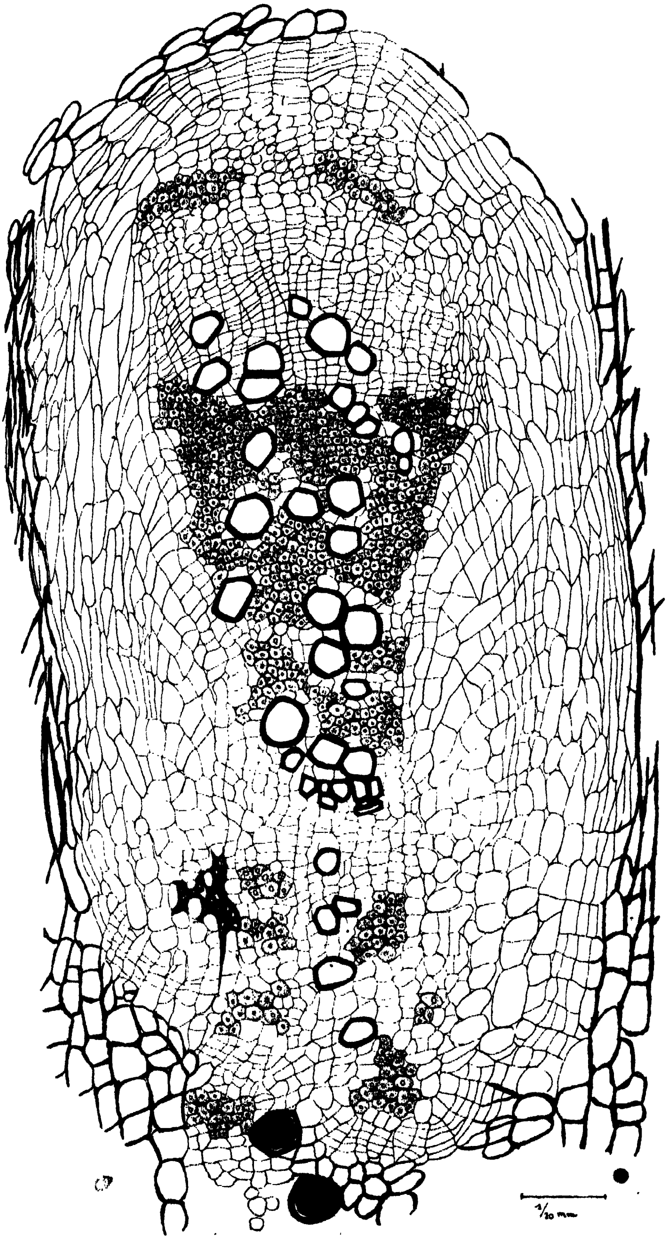


FIG. 11.—One vascular bundle from the upper part of the root of a hardy alfalfa plant whose bundles had been separated by killing of intervening parenchyma. The center of the root was hollow, surrounded by these separated bundles, each surrounded by meristematic tissue and separated from its neighbors by dead cells. Even though healed thus effectually, the plant was not thrifty

which may have been brought about by injury like that shown in Figure 6. Sometimes small local rifts in rays or elsewhere in parenchyma become filled with a gumlike material and no cell division follows.

In the mature wood there are two types of injury, the small brown spots arranged in circles (pl. 2, A) and the central injury. So far as the writer has been able to observe, the small spots are always surrounded sooner or later by thickened walls of the adjacent parenchyma or by the meristematic activity of such parenchyma so that decay does not spread to cause heart rot, as suggested by Weimer (7). Heart rot seems always to have its origin at the center of the plant. When the central tissue is killed during the first winter in the life of the plant, the necrotic cells can usually be completely separated from the healthy tissue by activity of the wood parenchyma, which is very abundant and is not interrupted by such large fiber masses as those in older growth. In older plants healing may be much more difficult. Not only do large fiber masses interrupt the parenchyma and by their slow decay continually extend the hollow center, but the mechanical action of swelling and shrinking of these woody masses with wetting and drying tends to tear apart the wood rays and expose new areas to fungous and bacterial invasion.

From the preceding it appears that the growth response to injury is conditioned by the character of the cells adjoining the injured tissue. If these cells are capable of prompt meristematic activity and the conditions surrounding the plant are favorable for growth, decay can not proceed far beyond the area originally killed. The loss of vascular tissue in the root does not appear to be a permanent handicap, and when new tissue has been produced abundantly, as shown in Plate 2, B, the plant continues growth with unabated vigor.

WINTER INJURY IN RELATION TO BACTERIAL WILT

During the last two years, in which winter injury has been seen in many fields in the central Mississippi Valley, much evidence has accumulated indicating that bacterial wilt (3) occurs in far greater abundance in such injured fields than in uninjured fields when sources of infection are approximately equal. Inasmuch as these parasitic bacteria require wounds through which to enter the plant, and since winter injury produces wounds apparently favorable for infection in the spring, when infection seems to take place most abundantly, it is highly probable that this injury is an important place of entry for the bacteria.

SUMMARY

A pathological condition of alfalfa roots and crowns caused apparently by adverse winter conditions has been described previously by Melchers and Weimer. The character of this injury is discussed in detail in this paper.

The development of the taproot and crown of alfalfa is traced and certain of their histological characteristics are described.

The injury in the plant tissue is found originating in characteristic locations in parenchyma of the phloem rays, in the central pithlike structure of the upper part of the taproot, and in the xylem.

In the spring of 1927 the injury appeared first as an apparent mechanical disorganization of tissue in plants taken from beneath an ice sheet. The healing of injuries in plants is traced through the spring.

Winter injury when severe appears not only to shorten the life of plants but to furnish a convenient point of entry for the parasitic bacterium (*Aplanobacter insidiosum* L. McC.) which causes bacterial wilt.

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A POSSIBLE RELATIONSHIP BETWEEN SOIL SALINITY AND STAND IN COTTON[1]

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INTRODUCTION

The problem of the factors influencing the germination of the seed and the establishment of the seedling is one of great interest in relation to succession in natural vegetation. It is of large economic importance not merely in the case of agricultural crop production but also in forest reproduction and in the reestablishment of native vegetation injured by overgrazing.

Relatively little seems to have been done in the application of statistical methods to the study of seedling stand[2] under field conditions.

The purpose of the present paper is to present the results of a first investigation of a possible relationship between soil salinity and seedling stand in Egyptian and upland cotton. The investigations on which this paper is based were conducted for the office of alkali and drought resistant crops, with the cooperation of the office of western irrigation agriculture and the seed laboratory of the Bureau of Plant Industry. They were carried out at the field station of the United States Department of Agriculture and the United States Indian Service at Sacaton, Ariz. While the results are not wholly consistent, they are so suggestive that it seems desirable to present them at this time because of their possible bearing on a number of important problems. Results of this kind are attained at such cost of labor that it is unlikely that another equally extensive series of observations can be secured and reduced to statistical constants at any time in the immediate future.

The specific problem undertaken was that of the possible correlation between the salinity of the soil and the number of seedlings established per unit area. The cotton plant is of particular interest for such investigations. Although cotton, like other crops, fails to produce a stand on extremely saline land, it is one of the most salt-resistant crops (*1*, *2*, *16*, *17*).[3] Investigations have shown that the chloride (*14*) and sulphate (*11*) content of the tissue fluids of Egyptian and upland cotton is high, but that there is differential absorption for these anions (*12*). This differentiation holds not merely for the American-bred Pima Egyptian cotton as compared with several upland varieties, but also for several Egyptian varieties

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[1] Received for publication July 2, 1928; issued October, 1928.

[2] The term "seedling stand," or "stand," rather than "germination rate" has been used because the census was made at the time when the plants had reached the stage of development at which thinning is usually done. It is possible that some of the seeds that had germinated might have died before this period.

[3] Reference is made by number (italic) to "Literature cited," p. 230.

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grown from newly imported seed (*13*). Evidence has also been adduced to show that there is a more rapid accumulation of chlorides by the Egyptian type.

All these facts suggest that the relation of the cotton seedling to soil salinity may be quite different from that of many other agricultural plants. In a study of this relationship in the Egyptian and upland types of cotton, interest is heightened by the fact that the tissue-fluid properties of these cottons have been shown to be very closely correlated with the salt content of the substratum on which they are grown (*8*). Interest is further increased by the fact that the distribution of the stand per hill actually secured in cotton grown on the distinctly saline soils of the Southwest has been shown to differ significantly from a random distribution. This deviation from a theoretical or chance distribution consists in the production of an excess of hills with few or no seedlings and of an excess of hills with a large number of seedlings on fields planted with a uniform number of seeds per hill. The significance of these deviations has been tested by Pearson's χ^2 criterion (*10*) and the amount of deviation measured in terms of Pearson's equivalent probability correlation coefficient (*15*).

MATERIALS AND METHODS

The cultural methods will be described, and then the methods of statistical analysis of the experimental data will be given in necessary detail.

CULTURAL METHODS

The data analyzed were obtained from two cultures, one grown in 1922 (experiment 3/22), and the other in 1923 (experiment 1/23) on the rather saline alluvial soil of the Gila River Valley, Sacaton, Ariz. (*10, 15*).

The experiment of 1922, designated as experiment 3/22 in this paper and elsewhere, was conducted on the north halves of plots E 3-1, E 3-2, and E 3-3 of the United States Field Station at Sacaton, Ariz. All three plots were treated exactly alike and may be regarded as a unit experimental plot 79.5 feet in width and 200 feet in length, planted to 24 rows of cotton. Ten feet at each end were devoted to buffer cultures. Each row was divided into 18 subrows each 10 feet in length and planted to 10 hills of cotton.

The plantings of each of the three varieties, Pima Egyptian (P) and Meade (M) and Acala (A) upland cottons, were distributed over the field as follows:

		(1)			(2)			(3)				(6)	
(1)	A	P	M	A	P	M	A	P	M	. . .	A	P	M
(2)	A	P	M	A	P	M	A	P	M	. . .	A	P	M
.		.			.			.				.	
.		.			.			.				.	
.		.			.			.				.	
(24)	A	P	M	A	P	M	A	P	M	. . .	A	P	M

The numbers in parentheses at the top represent sections and the numbers in parentheses at the left side represent rows. Each of these 10-foot sections planted to one variety may be designated as a plot. In consequence of this scheme of planting the three varieties are each distributed in 144 subrows or plots over the entire field.

If the field be heterogeneous with respect to soil salinity in the sense in which the term has been used in earlier investigations of the writer (*6*, *7*), and if the salinity of the soil has an influence on the germination rate or on the viability of the seedlings after germination, a correlation between the electrical resistance of the soil mass and the number of seedlings standing at a given time should be found.

Various phases of the problem of the physical heterogeneity of the field in the experiment of 1922 have already been treated. The results of these investigations show not merely that the field is heterogeneous with respect to soil properties as measured by the usual criterion (*6*), but also that this heterogeneity finds expression in the chloride content (*14*) and other physicochemical properties of the leaf-tissue fluids (*8*, *9*). They also show that the heterogeneity is itself a lawful phenomenon (*9*). They further show that the relationship between the properties of the soil solution and the physicochemical properties of the plant-tissue fluids may be measured directly in terms of the correlation coefficient or in terms of regression (*8*). In their bearing upon the present investigation these earlier results are of value in that they indicate the probable fruitfulness of the method of attack chosen.

In this experiment the soil samples were taken between rows 1 and 2, between 3 and 4, and so on, at three points approximately equally distributed over the 10-foot subrows devoted to each variety. This is exactly shown by a map published elsewhere (*9*). For the first triplet of the first four rows the arrangement is:

(1) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
* * * * * * * * * *
(2) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .

(3) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
* * * * * * * * * *
(4) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
. . . .
. . . .
. . . .
(24) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .

Here the letters A, P, M represent 10 individual plantings (hills) of each of the varieties shown in the scheme on page 214.

A combination of the three soil cores taken at the positions approximately indicated by the asterisks between each of the varieties of the two adjoining rows provided the soil samples necessary for the reading of the electrical conductivity of the saturated soil mass by means of the electric bridge described by Davis and Bryan (*3*). Determinations were reduced to the standard temperature of 60° F. by the table given by Davis and Bryan. Since rows 1 and 2 and subsequent pairs of rows are equally and but slightly separated from the series of borings made between them, the same soil sample may serve for the two rows.

Thus in this experiment there were 216 sets of soil conductivity determinations (72 for each variety), each comprising readings of four individual soil layers of 1-foot depth. The conductivities of these 72 sets of soil samples for each variety were weighted with the number of hills of the given variety in each two-row plot, which was 20 in each case. This makes a total of 1,440 hills, or a total of 72 plots weighted in a twofold manner, employed as a basis for the determination of correlations in each variety. Methods of weighting employed to eliminate the influence of wholly sterile hills will be indicated below.

In the experiment of 1923 (experiment 1/23) 16 rows on plots D 1–10 and D 1–11 of the United States field station, Sacaton, Ariz., were planted to alternate sections of Pima Egyptian and Lone Star upland cottons, and between each pair of these was planted 1 hill of hybrid cotton. Thus the order of planting was: 18 hills of Pima, 1 hill of hybrid, 18 hills of Lone Star, 1 hill of hybrid, 18 hills of Pima, etc. The 9 hills of Pima and the 9 hills of Lone Star cotton on either side of each hybrid plant may be considered as constituting an experimental unit for the purposes of the present study. There were 10 such units per row, which would therefore comprise 90 × 16 = 1,440 hills of each variety. The hybrid plants are not considered here.

Soil samples were taken between rows 1 and 2, between 3 and 4, between 5 and 6, and so on, in the manner indicated on page 215 for the scheme for the 1922 experiment, except that in this case the soil samples were taken in the immediate neighborhood of the hybrid plants.

Thus 80 sets of conductivities are available for both varieties. These have been weighted with the number of hills of each variety, which was 18 for the sections of the two adjoining rows, compared with the soils of each boring, or with the number of sections of rows, which was 2, in determining the correlations between soil resistance and seedling stand. A total of 1,440 hills is therefore involved, or a total of 80 plots weighted in a twofold manner. Methods of weighting employed in dealing with sterile hills will be indicated below.

METHODS OF STATISTICAL ANALYSIS OF DATA

In determining correlations between soil resistance and number of seedlings per hill the resistances were grouped in classes of 25-ohm range in the case of experiment 3/22. In experiment 1/23 classes of 15-ohm range were practicable. In determining the average resistance of the upper 4 feet of soil, the original resistances were first averaged and then grouped in classes of the range indicated for the individual feet of the two experiments.

The methods of determining the statistical constants will now be considered.

Let s be the number of seedlings per hill in each of the n hills of the N plots for which records of soil resistance, R, and seedling number are available. The problem is merely to determine the correlation between soil resistance, as an approximate measure of soil salinity, and number of seedlings germinating and surviving to the time of record.

In all the experiments six seeds were planted per hill. The number of seedlings correlated may therefore vary from none to six. It has been shown elsewhere (*10*, *15*) that there is reason for believing that special factors are involved in determining the complete absence of seedlings in the case of certain of the hills. It seems desirable, therefore, to consider the data in two classes, (1) those involving all hills; and (2) those involving hills in which at least one seedling was found at the time of thinning. Let n be the number of hills per plot and n' the number of hills per plot that produce at least one seedling. Let s denote the number of seedlings in the hills of the first class (none to six seedlings per hill) and s' the number of seedlings per hill in the second class (one to six seedlings per hill). The total number of hills involved in the correlations of the first class, $r_{Rs'}$, will be Nn. The total number of hills in correlations of the second class, r_{Rs}, will be $S(n')$, where S denotes summations for the several plots. In determining these correlations the soil resistance is weighted with the constant value of n in the first instance and with the variable value of n' in the second instance.

If the method of taking moments about 0 as origin (*4*, *5*) be employed,

$$\overline{R} = S(nR)/Nn \quad \text{.......} (1)$$

$$\overline{R}' = S(n'R)/S(n') \quad \text{.......} (2)$$

$$\sigma^2_R = \frac{S(nR^2)}{Nn} - \left(\frac{S(nR)}{Nn}\right)^2 \quad \text{.......} (3)$$

$$\sigma'^2_R = \frac{S(n'R^2)}{S(n')} - \left(\frac{S(n'R)}{S(n')}\right)^2 \quad \text{.......} (4)$$

where the primes denote constants due to weighting with the number of hills producing one or more seedlings, n', rather than with the constant number of hills planted per plot, n, and the bars and σ's denote means and standard deviations, respectively.

Let Σ denote summation within individual plots and S denote summation from plot to plot throughout the experimental field. Then $\Sigma(s') = \Sigma(s)$, $\Sigma(s'^2) = \Sigma(s^2)$, and for the whole population

$$\bar{s} = S[\Sigma(s)]/Nn = S[\Sigma(s')]/Nn \quad \text{.......} (5)$$

$$\bar{s}' = S[\Sigma(s')]/S(n') = S[\Sigma(s)]/S(n') \quad \text{.......} (6)$$

$$\sigma^2_s = \frac{S[\Sigma(s^2)]}{Nn} - \left(\frac{S[\Sigma(s)]}{Nn}\right)^2 = \frac{S[\Sigma(s'^2)]}{Nn} - \left(\frac{S[\Sigma(s')]}{Nn}\right)^2 \quad \text{.......} (7)$$

$$\sigma'^2_s = \frac{S[\Sigma(s'^2)]}{S(n')} - \left(\frac{S[\Sigma(s')]}{S(n')}\right)^2 = \frac{S[\Sigma(s^2)]}{S(n')} - \left(\frac{S[\Sigma(s)]}{S(n')}\right) \quad \text{.......} (8)$$

The correlations are:

$$r_{Rs}=\frac{\dfrac{S[R\Sigma(s)]}{Nn}-\left(\dfrac{S(nR)}{Nn}\cdot\dfrac{S[\Sigma(s)]}{Nn}\right)}{\sigma_R\sigma_s}$$

$$=\frac{\dfrac{S[R\Sigma(s')]}{Nn}-\left(\dfrac{S(nR)}{Nn}\cdot\dfrac{S[\Sigma(s')]}{Nn}\right)}{\sigma_R\sigma_s}\text{-----------}\tag{9}$$

$$r_{Rs}'=\frac{\dfrac{S[R\Sigma(s')]}{S(n')}-\left(\dfrac{S(n'R)}{S(n')}\cdot\dfrac{S[\Sigma(s')]}{S(n')}\right)}{\sigma'_R\sigma'_s}$$

$$=\frac{\dfrac{S[R\Sigma(s)]}{S(n')}-\left(\dfrac{S(n'R)}{S(n')}\cdot\dfrac{S[\Sigma(s)]}{S(n')}\right)}{\sigma'_R\sigma'_s}\text{-----------}\tag{10}$$

While the record of the number of seedlings surviving in a single hill may furnish some information concerning the properties of the soil as a factor influencing stand, it can not be a very reliable measure because of its demonstrably high variability.

Another and presumably better measure is furnished by the average production of the plot, $\Sigma(s)/n=\Sigma(s')/n$, or by the total number of seedlings per plot, say for convenience of notation $P=\Sigma(s)=\Sigma(s')$.

The constants of P are

$$\overline{P}=S[\Sigma(s)]/N=S[\Sigma(s')]/N\text{---------------}\tag{11}$$

$$\sigma^2_P=S\{[\Sigma(s)]^2\}/N-\{S[\Sigma(s)]/N\}^2=S\{[\Sigma(s')]^2\}/N-\{S[\Sigma(s')]/N\}^2\text{--}\tag{12}$$

and

$$r_{RP}=\frac{S[R\Sigma(s)]/N-\overline{R}\overline{P}}{\sigma_R\sigma_P}=\frac{S[R\Sigma(s')]/N-\overline{R}\overline{P}}{\sigma_R\sigma_P}\text{----------}\tag{13}$$

RESULTS

While a detailed discussion or description of the plots in biometric terms is unnecessary, it is desirable to show the mean values and the variabilities of soil resistance in the two experiments. These appear in Table 1. The constants here given are based on the actual number of soil samples analyzed. They are the constants used in determining the correlations between soil resistance and the number of seedlings per hill in the entire series, r_{Rs}, and between the soil resistance and the number of seedlings per plot, r_{RP}. The constants employed in the determination of the correlations for soil resistance and number of seedlings per hill in the selected series, which is limited to the hills that contained at least one seedling, are somewhat modified by weighting. These weighted values are not given, since all that is required for the purpose of the present discussion is a general idea of the order of magnitudes of the conductivities.

TABLE 1.—*Statistical constants for soil resistance at successive 1-foot depths, R_1 R_2, R_3, R_4, in plots at the United States Field Station, Sacaton, Ariz.*

Layers of soil	Mean	Standard deviation	Coefficient of variation
Experiment 3/22:			
Pima (Egyptian)—			
First foot, R_1	213. 19±4. 25	53. 52±3. 01	25. 10
Second foot, R_2	200. 00±7. 89	99. 30±5. 58	49. 65
Third foot, R_3	130. 56±6. 56	82. 52±4. 64	63. 21
Fourth foot, R_4	100. 35±4. 27	53. 76±3. 02	53. 58
First to fourth foot, $R_{1\text{-}4}$	160. 76±5. 15	64. 79±3. 64	40. 30
Meade (upland)—			
First foot, R_1	211. 80±4. 20	52. 86±2. 97	24. 96
Second foot, R_2	196. 18±7. 47	93. 97±5. 28	47. 90
Third foot, R_3	123. 61±5. 51	69. 33±3. 90	56. 09
Fourth foot, R_4	91. 67±3. 99	50. 17±2. 82	54. 73
First to fourth foot, $R_{1\text{-}4}$	153. 82±4. 74	59. 61±3. 35	38. 75
Acala (upland)—			
First foot, R_1	204. 17±4. 02	50. 52±2. 84	24. 74
Second foot, R_2	195. 14±7. 65	96. 25±5. 41	49. 32
Third foot, R_3	127. 78±6. 55	82. 45±4. 63	64. 52
Fourth foot, R_4	93. 75±3. 71	46. 72±2. 63	49. 84
First to fourth foot, $R_{1\text{-}4}$	154. 86±4. 96	62. 45±3. 51	40. 33
Experiment 1/23:			
Pima (Egyptian) and Lone Star (upland)—			
First foot, R_1	157. 88± . 89	49. 97± . 63	31. 65
Second foot, R_2	164. 81±1. 13	63. 08± . 80	38. 27
Third foot, R_3	145. 12±1. 08	61. 06± . 77	42. 07
Fourth foot, R_4	147. 75±1. 15	64. 52± . 81	43. 67
First to fourth foot, $R_{1\text{-}4}$	155. 06± . 95	53. 61± . 67	34. 57

The soil resistances are low, being of the general order of magnitude of 150 ohms at 60° F., thus indicating relatively high salinity. No attempt has been made to express these salinities in terms of the actual salt-content scale, since for purposes of comparison the original resistances probably represent a better measure of the soil solution than would any values computed from them.

A conspicuous feature of these results is that the general order of magnitude of the soil resistances in the two series is about the same. Salinities at the surface are somewhat higher in the experiment of 1923 than in that of 1922. Another conspicuous feature is the high variability of soil resistance from plot to plot. This variability is measured by standard deviations ranging from about 47 to about 99 ohms and by coefficients of variation ranging from about 25 to about 65 per cent. While these high variabilities are advantageous for the purposes of this investigation, since they show that the plots on which the experiments were carried out are highly heterogeneous, they also show that we are dealing with a phenomenon so variable that results of the highest precision can not be expected.

The means, standard deviations, and coefficients of variation of number of seedlings per hill in all hills (whole series), *s*; of seedlings per hill in the series selected to contain only hills producing at least one seedling (selected series), *s'*; and of number of seedlings per plot, *P*, appear in Table 2.

TABLE 2.—*Statistical constants for number of seedlings per hill and per plot in Pima, Meade, Acala, and Lone Star cottons as grown at the United States Field Station, Sacaton, Ariz., in 1922 and 1923*

Experiment and variety	Whole series (per hill)			Selected series (per hill)			Seedlings per plot		
	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation
Experiment 3/22:									
Pima (Egyptian)	1.98±0.03	1.94±0.02	98.19	3.08±0.03	1.57±0.02	50.88	19.76±0.61	10.82±0.43	54.75
Meade (upland)	1.31± .03	1.55± .02	117.87	2.38± .03	1.34± .02	56.54	13.12± .43	7.60± .30	57.98
Acala (upland)	2.08± .03	1.80± .02	86.61	2.91± .03	1.45± .02	49.94	20.78± .39	9.82± .39	47.27
Experiment 1/23:									
Pima (Egyptain)	1.17± .03	1.73± .02	148.59	2.85± .04	1.60± .03	56.07	10.51± .43	8.15± .31	77.55
Lone Star (upland)	1.05± .03	1.61± .02	152.96	2.70± .04	1.48± .03	54.87	9.45± .40	7.42± .28	78.50

The most conspicuous feature of this table is the low mean number of seedlings per hill. Six seeds were planted per hill in each case, but when all hills are included the average production is less than 1.5 seedlings per hill in three of the series and less than 2.25 seedlings per hill in the other two series. This is due primarily to the fact that a large number of hills produce no seedlings at all. This has been shown in the diagrams of an earlier paper (*10*). Even when hills producing no seedlings at all are excluded, as in the selected series, the average number of seedlings per hill reaches three in only one of the five series.

Thus it appears that in these experiments the stand produced was very poor. This is particularly true of experiment 1/23. It is quite possible that had all the conditions of growth been such as to produce on an average a more complete stand the correlation coefficients would have shown a more material magnitude and greater uniformity. Neither large nor uniform correlations can be expected with such high coefficients of variation as are characteristic of these experiments.

The variabilities, both absolute and relative, are of special interest. The coefficients of variation for number of seedlings per hill in the series in which hills producing no seedlings were included ranges from 87 to 153. When the calculations are based on the selected series, the standard deviations range from 50 to 56 per cent of the mean. When the variabilities of number of seedlings per plot are dealt with, the standard deviations range from 47 to 79 per cent of the average stand of these plots. It will be quite clear from these high coefficients that we are here dealing with a phenomenon so variable that smooth results can hardly be expected.

The correlations between soil resistance and seedling stand for the experiment of 1922, appear in Table 3, which shows the correlation between the soil resistance of the first to fourth foot, R_1 to R_4, and the number of seedlings germinating. The averages of these correlations are also given. These averages are not provided with probable errors, since it is not quite clear how these should be calculated. As a further measure of the relationship between the properties of the soil and the number of seedlings, the correlation between the average resistance of the soil of the first 4 feet, $R_{1\text{-}4}$, and the number of seedlings is also given. While there may be some question from both the physical and the biological standpoint as to the legitimacy of averaging the resistances of different layers of soil as a more general measure of the salinity of the soil mass as a whole, earlier studies (*8*) have shown that a closer correlation between soil properties and plant characteristics are thus secured.

The second column of Table 3 shows the relationship between soil resistance and the number of seedlings per hill (r_{Rs}) when all hills are included, irrespective of whether they produced seedlings. The fourth column shows the correlation between soil resistance and the number of seedlings per hill ($r_{Rs'}$) for hills that contain at least one seedling. The sixth column shows the difference between these two coefficients. The probable errors of these differences have been determined without regard to possible correlation between the two correlations. They are, therefore, probably too large. The eighth column shows the correlations between soil resistance and the total number of seedlings per plot (r_{RP}).

TABLE 3.—*Correlation coefficients measuring the relationship between soil salinity in terms of the electrical resistance of the soil mass and seedling stand in Egyptian and upland cottons grown at the United States Field Station, Sacaton, Ariz., in 1922*

Depth of sample	Correlation between soil resistance and number of seedlings per hill (whole series) r_{Rs}		Correlation between soil resistance and number of seedlings per hill (selected series) r_{Rs}'		Difference between correlation in whole series and selected series $r_{Rs}-r_{Rs}'$		Correlation between soil resistance and number of seedlings per plot r_{RP}'	
	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	*Diff.* $\pm E_{diff.}$	*Diff.*$/E_{diff.}$	$r \pm E_r$	r/E_r
Pima (Egyptian):								
First foot, R_1	−0.2048 ± .0170	12.03	−0.0975 ± .0220	4.44	−0.1073 ± .0278	3.86	−0.3490 ± .0494	7.07
Second foot, R_2	− .2002 ± .0171	11.74	− .0847 ± .0220	3.84	− .1155 ± .0279	4.14	− .3476 ± .0494	7.03
Third foot, R_3	− .1617 ± .0173	9.34	− .0690 ± .0221	3.12	− .0927 ± .0281	3.30	− .2812 ± .0518	5.43
Fourth foot, R_4	− .1440 ± .0174	8.27	− .0813 ± .0221	3.69	− .0627 ± .0281	2.23	− .2494 ± .0527	4.73
Average correlation	− .1777	.....	− .0831	.....	.....	.....	− .3068	.....
First to fourth foot, R_{1-4}	− .2062 ± .0170	12.12	− .1020 ± .0220	4.64	− .1042 ± .0278	3.75	− .3563 ± .0491	7.26
Meade (upland):								
First foot, R_1	− .1788 ± .0172	10.40	− .1084 ± .0236	4.59	− .0704 ± .0292	2.41	− .3006 ± .0511	5.88
Second foot, R_2	− .1506 ± .0174	8.67	− .0955 ± .0237	4.03	− .0551 ± .0294	1.87	− .2802 ± .0518	5.41
Third foot, R_3	− .1198 ± .0175	6.84	− .0750 ± .0238	3.15	− .0448 ± .0295	1.52	− .2213 ± .0535	4.14
Fourth foot, R_4	− .0999 ± .0176	5.68	− .0742 ± .0238	3.12	− .0257 ± .0296	.87	− .1771 ± .0544	3.25
Average correlation	− .1373	.....	− .0883	.....	.....	.....	− .2448	.....
First to fourth foot, R_{1-4}	− .1531 ± .0174	8.82	− .1067 ± .0236	4.51	− .0464 ± .0293	1.58	− .2756 ± .0519	5.30
Acala (upland):								
First foot, R_1	− .1577 ± .0173	9.10	− .0559 ± .0210	2.67	− .1018 ± .0272	3.74	− .3448 ± .0495	6.96
Second foot, R_2	− .1048 ± .0176	5.96	− .0123 ± .0210	.58	− .0925 ± .0274	3.38	− .2058 ± .0538	3.82
Third foot, R_3	− .1147 ± .0175	6.54	− .0315 ± .0210	1.50	− .0832 ± .0273	3.05	− .2268 ± .0533	4.25
Fourth foot, R_4	− .1205 ± .0175	6.88	− .0634 ± .0210	3.03	− .0571 ± .0273	2.09	− .2408 ± .0530	4.55
Average correlation	− .1244	.....	− .0408	.....	.....	.....	− .2546	.....
First to fourth foot, R_{1-4}	− .1526 ± .0174	8.79	− .0553 ± .0210	2.63	− .0973 ± .0273	3.56	− .3061 ± .0509	6.01

A glance at Table 3 shows that the coefficients are negative in sign throughout. Since soil salinity is measured in terms of electrical resistance in ohms, the negative values of these coefficients indicate that in this experiment higher soil salinities are accompanied by higher seedling stands.

The first measure of interrelationship (the correlations between soil resistances and number of seedlings in all hills, r_{Rs}) shows that the coefficients are from 5.68 to 12.12 times as large as their probable errors. There can, therefore, be no question that in this series of determinations the more saline portions of the field, which are characterized by lower electrical resistances, produce a larger seedling stand. The average correlations are of the order -0.124 (Acala upland) to -0.178 (Pima Egyptian). The correlations for the average resistance of the first to fourth foot are larger than the average correlations, but not larger than all of the individual constants for the several soil layers.

Correlations between soil resistance and the number of seedlings per hill in the series comprising only hills that produced at least one seedling ($r_{Rs'}$) are negative throughout, but are smaller in comparison with their probable errors than are the preceding constants. Their ratios to their probable errors, which have been calculated by the usual formula, range from 0.58 to 4.64.

The algebraic differences between these correlations and those based on all of the hills (including sterile hills) are negative throughout. They show that the correlations are more negative when the vacant hills are included than when the coefficients are based on a selected series of hills each including at least one seedling. It may be noted, however, that inasmuch as all signs are negative, the algebraic differences will differ only in sign from the numerical differences in the same direction, which are consistently positive. The significance of the numerical values of the differences therefore offers no complication.

The fact that the coefficients as determined by both methods are consistently negative would seem to indicate that under the conditions of this experiment the salinity of the soil has such a definite influence upon the seedling stand that a more perfect stand is produced on sections of the field having higher soil salinity. This conclusion is strengthened by the fact that the inclusion of the hills which produced no seedlings increases the magnitude of the correlation.

The correlations between the resistance of the soil mass and the total number of seedlings per plot are in every instance conspicuously higher than either of the preceding values. They range from -0.177 to -0.356, with average values of -0.307 for Pima Egyptian, -0.245 for Meade upland, and -0.255 for Acala upland cotton. These averages are based on the coefficients determined from the upper 4 feet of soil, and do not include the coefficients based on the average resistance of these 4 feet. No probable errors are given for these averages, since only four cases are involved.

The correlations for the average resistance of the upper 4 feet of soil and the seedling stand produced are of the same order of magnitude as the average of the correlations for the individual soil layers and seedling stand. These coefficients are from 3.25 to 7.26 times as large as their probable errors. Thus there can be no question concerning their individual trustworthiness.

It is interesting, and possibly significant, that in general the correlations between the resistances of the upper foot or the upper 2 feet of soil and seedling stand are higher than those for the lower layers of soil. This is exactly what might be expected if the salinity of the soil directly influenced seedling stand. It is important to note, however, that the soil samples were taken near the end of the season, after the tissue-fluid determinations with which they have also been correlated (*8*) were made, and not at the exact time that seedling stand was being established. Doubtless some vertical movements of salts occurs with the march of the season.

Since the values of the correlation coefficients are low, it is reasonable to expect considerable irregularity in the distribution of the empirical means about the regression lines. Nevertheless, it has seemed desirable to determine the straight-line equations showing the relationship between number of seedlings per hill and soil salinity in the total series and in the selected series.

The straight-line equations showing the regression of the number of seedlings per hill on the soil resistance for the three varieties of cotton appear in Table 4. The lines for Pima Egyptian cotton are represented in Figure 1, those for Meade upland cotton in Figure 2, and those for Acala upland cotton in Figure 3.

TABLE 4.—*Regression of number of seedlings per hill on soil resistance, R, for Pima, Meade, and Acala cottons as grown at the United States Field Station, Sacaton, Ariz., in 1922*

Layers of soil	Regression for Pima (Egyptian)	Regression for Meade (upland)	Regression for Acala (upland)
First foot, R_1	$s = 3.5567 - 0.0074\ R_1$ $s' = 3.6611 - .0028\ R_1$	$s = 2.4198 - 0.0052\ R_1$ $s' = 2.9174 - .0027\ R_1$	$s = 3.2255 - 0.0056\ R_1$ $s' = 3.2241 - .0016\ R_1$
Second foot, R_2	$s = 2.7570 - .0039\ R_2$ $s' = 3.3449 - .0014\ R_2$	$s = 1.7979 - .0025\ R_2$ $s' = 2.6385 - .0014\ R_2$	$s = 2.4609 - .0020\ R_2$ $s' = 2.9477 - .0002\ R_2$
Third foot, R_3	$s = 2.4710 - .0038\ R_3$ $s' = 3.2561 - .0014\ R_3$	$s = 1.6422 - .0027\ R_3$ $s' = 2.5508 - .0015\ R_3$	$s = 2.3984 - .0025\ R_3$ $s' = 2.9823 - .0006\ R_3$
Fourth foot, R_4	$s = 2.4960 - .0052\ R_4$ $s' = 3.3219 - .0025\ R_4$	$s = 1.5940 - .0031\ R_4$ $s' = 2.5648 - .0022\ R_4$	$s = 2.5137 - .0046\ R_4$ $s' = 3.0968 - .0020\ R_4$
First to fourth foot, R_{1-4}	$s = 2.9672 - .0062\ R_{1-4}$ $s' = 3.4799 - .0026\ R_{1-4}$	$s = 1.9225 - .0040\ R_{1-4}$ $s' = 2.7404 - .0025\ R_{1-4}$	$s = 2.7596 - .0014\ R_{1-4}$ $s' = 3.1088 - .0013\ R_{1-4}$

In these figures the lower lines, with empirical means represented by circles, show the regression of number of seedlings per hill on soil resistence in the complete series. The upper lines, with empirical means represented by solid dots, show the regression of the number of seedlings per hill on soil resistance in the selected series. Since the mean number of seedlings per hill in the selected series is higher than that in the whole series (because of the omission of hills with no seedlings) the lines for the selected series lie above those for the whole series.

All the regression lines show only slight slopes, as is to be expected from the low values of the correlation coefficients. While the empirical means are distributed with considerable irregularity about the lines, there can be no question that a straight-line equation represents the results as well as they can be graduated by any one equation. This result lends confidence to the writer's conclusion concerning the reality of the existence of a negative correlation between soil resistance and seedling stand in the 1922 experiment, on which the conclusions of the present paper are primarily based.

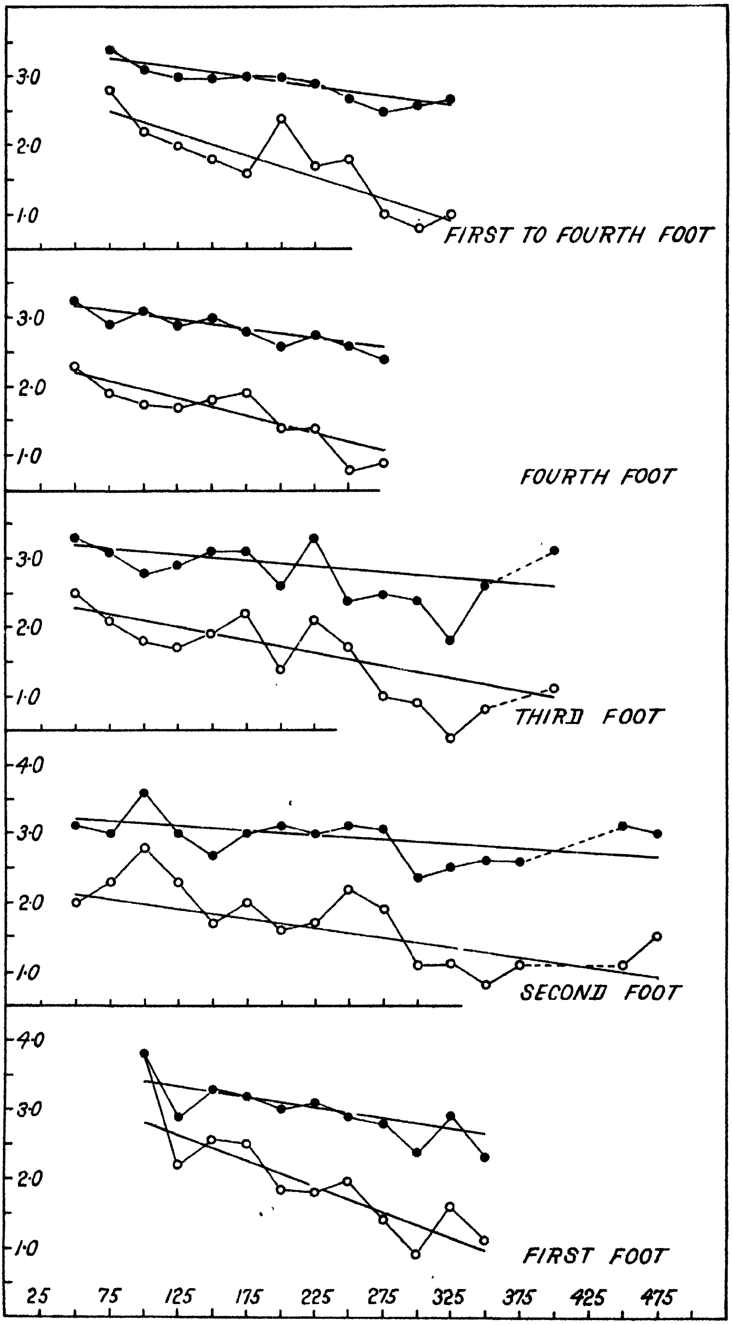

FIG. 1.—Regression of number of seedlings per hill in Pima Egyptian cotton on soil resistance in the 1922 experiment. Lower lines with the empirical means (circles) represent the whole series. Upper lines with empirical means (solid dots) represent the selected series

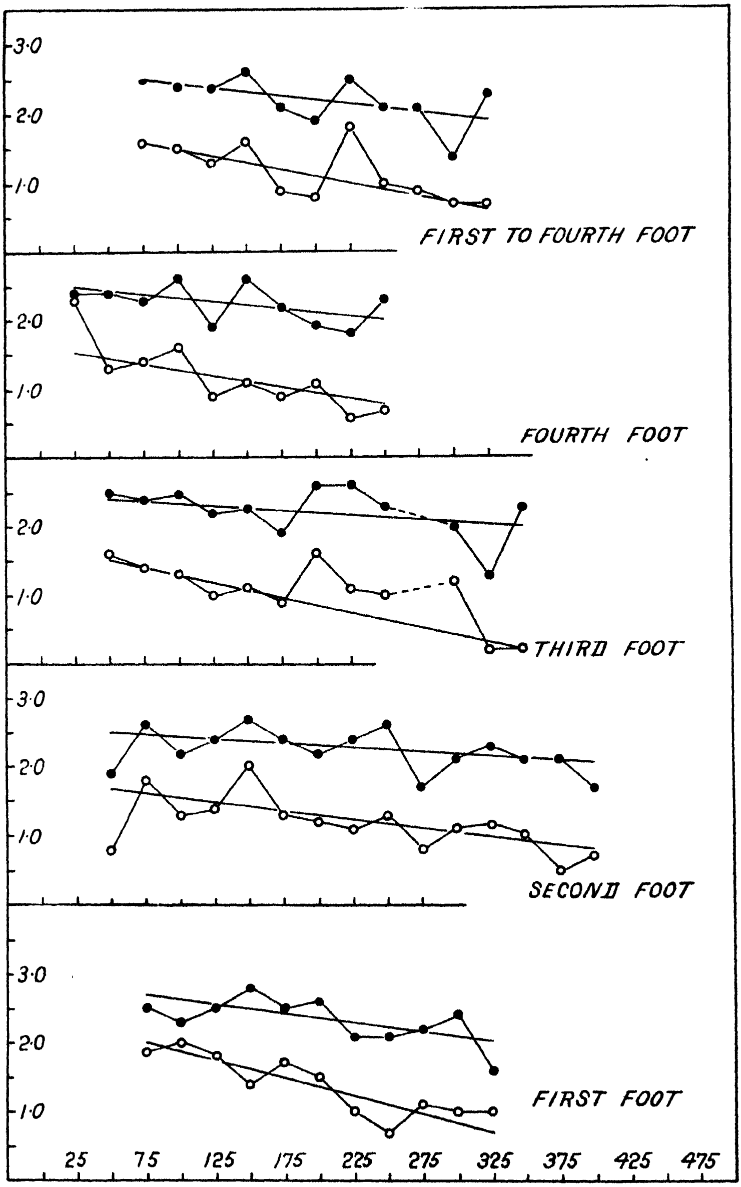

FIG. 2.—Regression of number of seedlings per hill in Meade upland cotton on soil resistance in the 1922 experiment. Lower lines with the empirical means (circles) represent the whole series. Upper lines with empirical means (solid dots) represent the selected series

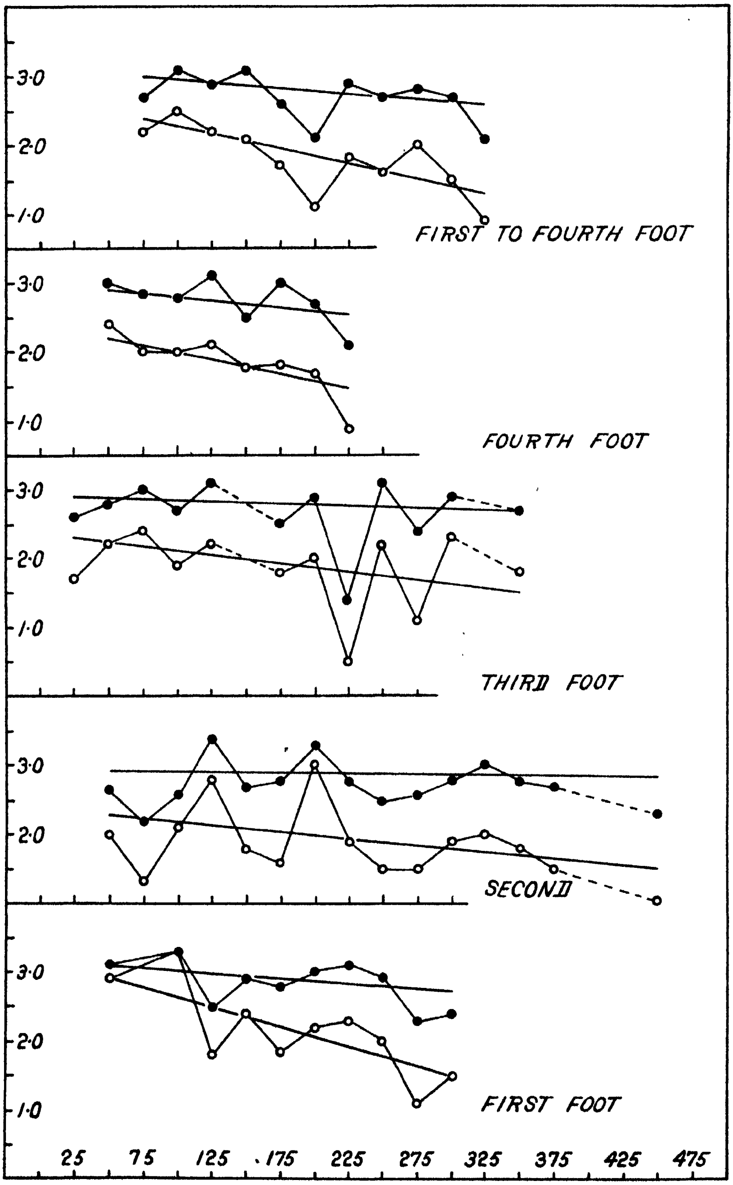

FIG. 3.—Regression of number of seedlings per hill in Acala upland cotton on soil resistance in the 1922 experiment. Lower lines with the empirical means (circles) represent the whole series. Upper lines with empirical means (solid dots) represent the selected series

The correlation coefficients showing the relationship between soil resistance and number of seedlings per hill and per plot in the series of determinations of the 1923 experiment appear in Table 5, which is comparable with Table 3.

TABLE 5.—*Correlation coefficients measuring the relationship between soil salinity in terms of the electrical resistance of the soil mass and seedling stand in Egyptian and upland cottons grown at the United States Field Station, Sacaton, Ariz., in 1923*

Depth of sample	Correlation between soil resistance and number of seedlings per hill (whole series) r_{R_s}		Correlation between soil resistance and number of seedlings per hill (selected series) $r_{R_s'}$		Difference between correlation in whole series and selected series $r_{R_s} - r_{R_s}'$		Correlation between soil resistance and number of seedlings per plot r_{RP}	
	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$Diff. \pm E_{diff}$	$Diff./E_{diff.}$	$r \pm E_r$	r/E_r
Pima (Egyptian):								
First foot, R_1	+0.0741 ±.0177	4.19	+0.0538 ±.0277	1.94	+0.0203 ±.0329	0.62	+0.1419 ±.0522	2.72
Second foot, R_2	−.0318 ±.0179	1.78	−.0220 ±.0278	.79	−.0098 ±.0331	.30	−.0614 ±.0535	1.15
Third foot, R_3	−.0572 ±.0177	3.23	−.0416 ±.0277	1.50	−.0156 ±.0335	.46	−.1096 ±.0527	2.08
Fourth foot, R_4	−.0587 ±.0177	3.32	−.0321 ±.0277	1.16	−.0266 ±.0329	.81	−.1125 ±.0526	2.14
Average correlation	−.0184		−.0105				−.0354	
First to fourth foot, R_{1-4}	−.0178 ±.0178	1.00	−.0141 ±.0278	.51	−.0037 ±.0330	.11	−.0342 ±.0533	.64
Lone Star (upland):								
First foot, R_1	+.0204 ±.0178	1.15	+.0661 ±.0284	2.33	−.0457 ±.0355	1.36	+.0398 ±.0532	.75
Second foot, R_2	−.0723 ±.0178	4.06	+.0770 ±.0283	2.72	−.1493 ±.0334	4.47	−.1420 ±.0526	2.70
Third foot, R_3	+.0076 ±.0178	.43	+.1487 ±.0278	5.34	−.1411 ±.0330	4.28	+.0148 ±.0533	.28
Fourth foot, R_4	−.0300 ±.0178	1.68	+.0689 ±.0283	2.43	−.0989 ±.0334	2.96	−.0584 ±.0531	1.10
Average correlation	−.0186		+.0902				−.0364	
First to fourth foot, R_{1-4}	−.0161 ±.0178	.91	+.1089 ±.0281	3.87	−.1250 ±.0333	3.75	−.0314 ±.0533	.59

The coefficients are low and extremely irregular in magnitude. In part they are positive and in part negative in sign. In general they can not be considered significant in comparison with their probable errors.

Considering first the correlation between the soil resistance and number of seedlings per hill in the whole series, r_{R_s}, we note that for Pima Egyptian cotton four of the correlations are negative and one is positive. For Lone Star upland cotton three of the coefficients are negative and two are positive. One of the positive coefficients (that for the first foot in Pima Egyptian cotton) may be significant in comparison with its probable error. The other two are certainly insignificant. The averages are slightly negative for both Pima Egyptian and Lone Star upland cottons.

The correlations between soil resistance and number of seedlings per hill in the series selected to contain only hills with at least one seedling are slightly positive in the Lone Star series but are negative in four of the five determinations for Pima Egyptian cotton. In this case the significance of the differences between the two series of correlations offers some complication. The differences in the table are algebraic and are consistently negative with the exception of that for the first foot in the Pima Egyptian series. The numerical differences in the same direction $(r_{Rs} - r_{Rs}')$ are consistently positive for Pima and consistently negative for Lone Star.

The correlation between soil resistance and seedlings per plot are negative in 7 of the 10 cases. They are, however, low in magnitude.

The diversity of signs and the low numerical values of the correlation coefficients in this experiment show that no final conclusions can be drawn from it. There is some slight support for a positive correlation between soil salinity and seedling stand, as was so clearly indicated by the results of experiment 3/22, but these evidences are so slight as to be of little value.

The significance of these results will be discussed below.

DISCUSSION OF RESULTS

The results of the foregoing experiments are inconsistent in that the first, that of 1922, indicates a low but consistent and statistically significant relationship between the salinity of the soil and seedling stand in the case of Pima Egyptian, Meade upland, and Acala upland cottons, whereas the second, that of 1923, furnishes no conclusive evidence for the existence of such a relationship.

This inconsistency of results might at first thought seem to throw serious doubt upon the existence of any relationship between soil salinity and seedling stand. It unquestionably shows that final conclusions must await further investigations. It is unfortunate that the evidence of further series can not be presented immediately. Such experiments are, however, exceedingly laborious. It is unlikely that they can be completed in the near future. It seems proper, therefore, to present the constants derived from these two series, to suggest reasons for the inconsistency of the findings, and to offer possible interpretations of the results.

First of all, it must be noted that we are here dealing with highly variable characters. Seedling stand is on an average low in both of the available experiments and is presumably influenced by a number of factors. High correlations can not, therefore, be expected without the closest possible control of all conditions.

The results of the first experiment are wholly consistent throughout. If this were the only experiment available the existence of a definite relationship between soil salinity and seedling stand would be considered fully demonstrated. It is the belief of the writer that the inconclusive results of the second experiment are due primarily to its being technically less reliable than the first, and that it should be essentially disregarded in the drawing of conclusions. It has been included here merely because it is improper to suppress data that fail to support the conclusions drawn.

The 1922 experiment was carried out with the greatest care and in the greatest detail with a view to securing soils in immediate association with the plants. The soil borings were uniformly distributed over each of the 10-foot plots so that the soils were sampled very close to the seedlings, although at a date later than that at which the seedling records were made. In the 1923 experiment the soil samples were taken between the rows on either side of a group of hybrid plants that occupied a position between the upland and Egyptian plots. Thus the soil samples are unquestionably less representative of either of the two varieties with which they are correlated than is the case in the 1922 experiment.

The question as to why the soil samples were not so accurately distributed with reference to the Pima Egyptian and Lone Star upland plants of the 1923 experiment as with reference to the Pima Egyptian and Meade and Acala upland plants of the 1922 experiment is pertinent. The answer is twofold: First, the 1923 experiment was planned primarily for a comparison of hybrid plants with the parent types lying immediately on either side of the hybrid group. It was not planned specifically with a view to determining the correlations between each of the parental types individually and the soil resistance of the immediate substratum on which the seedlings were produced. Second, the 1923 experimental work was carried out far in advance of the completion of the statistical analysis of the data secured in 1922. At the time the samples were taken in 1923 there was no reason to suspect that the relationship between the concentration of the soil solution and the characteristics of the plants would be so definite as it was subsequently demonstrated to be. There was no reason for believing that under field conditions such high correlations could be demonstrated as those shown in the investigation of the relationship between the concentration of the soil solution and the physicochemical properties of the plant-tissue fluids as was subsequently demonstrated on the basis of the experimental results of 1922 (*8*).

Thus the soil sampling of 1923 was carried out merely to obtain some general measure of the soil heterogeneity of this field and its relation to the plant. In consequence the records do not, in the opinion of the writer, represent as accurate or as valuable data for the purposes of this investigation as do those of 1922.

On a priori grounds the correlation between seedling stand and soil salinity under field conditions suitable for crop production would be expected to be low. Upon laying the correlations between soil resistance and seedling stand beside those for soil resistance and tissue-fluid properties as measured in 1922, we find that in that experiment the correlations for seedling stand are very small as compared with those for tissue-fluid properties. Under these conditions it is reasonable to suppose that a relationship between seedling stand and soil salinity would be demonstrable only under ideal technical conditions. Evidence that the conditions of 1923 were not ideal for the purpose of the phases of the investigation here under consideration is furnished by the fact that unpublished correlations between soil resistance and tissue-fluid properties in the 1923 experiment are lower than those in the 1922 experiment.

In view of the foregoing facts it seems quite probable that the failure to obtain correlations between soil resistance and seedling stand in 1923 comparable to those secured in 1922 may have been due to the experimental data being less suitable for the purposes of the investigation.

This comparison of results obtained in the two series is not without value in indicating the great care that is necessary in the execution of the technical phases of investigations of this kind.

The physical and physiological explanation of these results must be a subject for further investigation. It is probable that a factor of great importance in determining seedling stand in cotton may be the texture of the soil mass overlying the planted seeds. It is possible that in the more saline soils the seedling encounters less difficulty in breaking through the soil layer than in the less saline soils. It is also possible that plants grown under the more saline conditions are less subject to the attack of some diseases. Both of these factors must be further investigated. Peculiarities of the frequency distributions of the numbers of seedlings produced per hill (*10*, *15*) indicate that some such factors must be operative.

SUMMARY

The present investigation has dealt with the problem of the relationship between the concentration of the soil solution and the seedling stand produced in cultures of Pima Egyptian and of Meade, Acala, and Lone Star upland cotton on heterogeneous experimental fields.

Soil salinity was measured in terms of the electrical resistance of the saturated soil mass in the standard soil bridge cup. Seedling stand was recorded in terms of number of seedlings produced per hill in hills planted with a uniform number of seeds (six per hill), in terms of numbers of seedlings per hill in hills producing at least one seedling, and in terms of number of seedlings per plot, each plot comprising an equal number of hills.

The data for an experiment conducted in 1922, which was admirably designed to throw light on this problem, indicate that there is a significant negative correlation between soil resistance and seedling stand. Since soil resistance is measured in ohms, and therefore increases with diminishing salt content, this negative correlation indicates that under the range of soil salinities and other conditions of the experiment better stands are produced on more saline soils.

These relationships should not be expected to hold if cotton were planted on soils of indefinitely higher salt content. They may be typical of soils having the degree of salinity here involved.

The results of an experiment made in 1923 do not fully confirm those of the 1922 experiment. It is the belief of the writer that the 1923 experiment was technically inadequate for an investigation of this kind. The results are included merely to avoid bias in the presentation of data.

Assuming that under certain ranges of field conditions there is a positive correlation between the concentration of the soil solution and the seedling stand of cotton (or, as here expressed, a negative corrrelation between the electrical resistance of the soil solution as measured in the soil and seedling stand), further problems as to the physical, chemical, physiological and perhaps pathological causes of this relationship are presented for solution.

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A SURVEY OF THE RESISTANCE OF SUBSPECIES OF BRASSICA OLERACEA TO YELLOWS (FUSARIUM CONGLUTINANS)[1]

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INTRODUCTION

The wild cabbage (*Brassica oleracea* L.) is a native of the Old World, where it is found on the sea cliffs of western and southern Europe. From this leafy biennial or its progenitor have been derived presumably the cabbage (*B. oleracea* var. *capitata* L.), kale and collard (*B. oleracea* var. *acephala* DC.), cauliflower and broccoli (*B. oleracea* var. *botrytis* L.), Brussels sprouts (*B. oleracea* var. *gemmifera* DC.), and kohl-rabi (*B. oleracea* var. *caulo-rapa* DC.).[2] The organism (*Fusarium conglutinans* Woll.) causing yellows of this group of plants is apparently native to America and so far has not been reported from the Old World. Though first described as a disease of cabbage, it has been found on other forms of *B. oleracea*, and the data presented herewith show that the wild form and nearly all the varieties of the cultivated subspecies tested are more or less susceptible. Two reports of its occurrence on other species of Brassica have been made. According to Melhus,[3] Gilman found it upon the Chinese cabbage (*B. pekinensis* Rupr.) and Gregory reported it upon turnip (*B. rapa* L.).[4] The writers have never found it on the last two species, although they have included them in trials on infested soils where the disease developed abundantly on susceptible forms. The varieties of cabbage and its related forms have been developed largely in Europe out of contact with yellows and where natural or artificial selection for resistance through exposure to the disease could not have occurred.

The successful control of cabbage yellows has been based upon the fact that in every commercial variety so far tested at least a few individuals are highly resistant under field conditions. The selection of highly resistant strains from such individuals of several standard varieties has already been described.[5] [6] Although none of

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[2] SINSKAJA, E. THE ORIGIN OF THE VARIETIES OF THE CABBAGE TRIBE AND THE BASIS OF THEIR CLASSIFICATION. Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) 17 (4): 351-390. 1927. [In Russian.]

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[5] JONES, L. R., and GILMAN, J. C. THE CONTROL OF CABBAGE YELLOWS THROUGH DISEASE RESISTANCE Wis. Agr. Expt. Sta. Research Bul. 38, 70 p., illus. 1915.

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the varieties popular in America seems to possess sufficient resistance in itself to be of commercial value for use on yellows-infested soil, no exhaustive study has been made of the degree of natural resistance, especially of varieties used commonly in Europe but seldom in America. The fact that Jones and Gilman [7] found two varieties, the Volga or Stonehead and the Houser, that possessed a marked degree of resistance leads one to expect that other such varieties might be found.

No previous study of the varietal differences in resistance among forms other than cabbage has been made. In connection with the program of selection of yellows-resistant varieties of cabbage it has been a matter of scientific and practical value to give some attention to the relative resistance not only among a larger number of cabbage varieties but also among the varieties of cabbage relatives. This paper is a report of such investigations, which have been under way since 1924.

METHODS

The method of testing was similar to that already described in connection with the trials of cabbage strains selected for resistence.[8] The test plot of soil, which is located in eastern Kenosha County, Wis., has become, through repeated cropping with cabbage, as uniformly and thoroughly infested with the yellows organism as possible under natural conditions. The seed was sown upon yellows-free soil about May 15, and the plants were transplanted to the infested soil during the first week of July. They were inspected several times during the season, and each plant that showed any sign of yellows was marked permanently with a bamboo stake. The symptoms of the disease on the cabbage relatives are so similar to those on cabbage that special description here seems unnecessary. As in cabbage, the severity of attack commonly varies among individuals of a given variety. Part of this variation may be due to environmental influences or variation in distribution of the pathogene, and part to hereditary differences, but these points can be settled only by further study. Some plants are killed rapidly; others are decidedly stunted, but not killed; others show very slight injury; and still others show traces of yellows in midseason on lower leaves that soon drop off, but mature with no apparent setbacks. From the practical standpoint one is interested in the total damage done by the disease, which obviously is not always truly represented when only the percentage of total plants showing yellows is given. At the end of the season, therefore, the affected plants were divided into two lots: (1) Those killed or severely injured, and (2) those showing only slight injury or apparently complete recovery.

The number of plants used for the test of a given variety was in some cases less than 25. This number may appear too small to compensate for variations in soil infestation. Larger trials and numerous replications are of course desirable, but time and space did not permit more extensive planting when so many lots were to be tested. Repeated use of the soil in question for similar purposes has given such consistent results that confidence in the reasonable accuracy of the method is sustained. Further weight is given the results by the repetition of many of the trials during two successive seasons.

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[7] JONES, L. R., and GILMAN, J. C. Op. cit.

[8] WALKER, J. C., MONTEITH, J., Jr., and WELLMAN, F. L. Op. cit.

RESULTS

CULTIVATED CABBAGE

The results secured with cabbage varieties are divided into two groups, those used commonly in the United States and those used chiefly in Europe. In Table 1 is found, first, the behavior of four varieties of cabbage selected for resistance. It will be seen that little severe injury was noted, although a small percentage of each variety showed some evidence of the disease. Second, the results are given of tests with a number of lots of Volga or Stonehead and Houser, the two varieties earlier noted by Jones and Gilman [9] to possess some natural resistance. With the exception of one lot of Houser, all of these are fairly resistant, although less so than the varieties selected for resistance. Third, the reactions of a number of the varieties more commonly used in America are shown. All these, in contrast with the other two groups, are very susceptible and quite obviously of no value for use upon infested soil.

TABLE 1.—*Comparative Fusarium resistance of a number of cabbage varieties commonly used in the United States, including varieties selected for resistance*

Degree of resistance	Variety	1925 trials			1926 trials		
		Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered	Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered
			Per cent	*Per cent*		*Per cent*	*Per cent*
Resistant (selected)	Wisconsin Hollander	100	1	6	25	0	20
	Wisconsin All Seasons	100	1	7	50	0	6
	Wisconsin Brunswick	50	0	16	25	0	16
	Maryland Flat Dutch	52	0	4	25	0	8
Moderately resistant (natural) [a]	Volga	25	16	36			
					24	8	33
					15	0	47
					23	13	13
	Early Stonehead [b]				24	21	20
	Late Stonehead [b]				34	21	41
	Houser	25	28	32			
					25	16	24
					24	54	21
					24	17	46
Susceptible	Jersey Wakefield	72	65	17			
	Charleston Wakefield	246	64	20			
	Copenhagen Market	251	78	9	44	73	0
	All Head Early	112	54	37	51	61	14
	Succession				192	51	27
	All Seasons				47	55	23
	Danish Ballhead	191	73	23	65	65	12
	Haco (red)	256	79	12	246	58	13

[a] Samples of each from several sources.

[b] The Stonehead variety is very similar to if not identical with the Volga.

In Table 2 are given the data from trials of a number of varieties seldom used in America but commonly listed by seedsmen in one or another part of the Old World. Most of these varieties came from England and France but two came from Egypt. Most of them differ from the varieties in general use in America and in the north of Europe in that they produce loose rather than compact heads. The 1925 trials showed many of them to be very susceptible, and only those showing distinct resistance were tested again in 1926. They are grouped roughly into three lots. In the first group are those that are very susceptible and fall into a class with the average commercial variety used in America. In the second group are 12 varieties that are moderately resistant. Many of these compare favorably with the Houser and Volga varieties. In the third group are 7 varieties that are rather highly resistant and could be used with commercial success on infested soil. Though all are not quite in a class with the varieties selected for resistance (Table 1), St. Denis and Vaugirard d'Hiver compare very favorably with them. None of these varieties, however, are of the type suitable for culture in America.

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[9] JONES, L. R., and GILMAN, J. C. Op. cit.

The evidence is sufficient to show that there is a wide range of resistance among cabbage varieties that have been developed, so far as is known, out of contact with the yellows organism. If it is true that cultivated cabbage has evolved from the present known wild form without opportunity for natural selection for resistance to *Fusarium conglutinans*, the conditions found are what might be reasonably expected, since, as is shown later in this paper, the wild form is apparently heterozygous for the resistance character. It is perhaps a mere coincidence that those cultivated varieties of cabbage that became popular in American culture were of the very susceptible sorts, while certain others that might have been introduced except for other undesirable features would not have been jeopardized by yellows.

TABLE 2.—*Comparative Fusarium resistance of a number of cabbage varieties in general use in the Old World but seldom grown in the United States*

Degree of resistance	Variety	Source of seed	1925 trials			1926 trials		
			Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered	Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered
				Per cent	*Per cent*		*Per cent*	*Per cent*
Very susceptible	Joanet Hatif	France	24	88	0			
	All Heart	England	24	63	4			
	Offenham	do	25	60	0			
	Express	do	25	52	28			
	Late Drumhead Savoy	do	25	52	20			
	Etamps	France	25	52	12			
	Charentais Tardif	do	24	50	29			
	Blanc d'Hiver	do	24	50	17			
	Milan Cressonier	do	25	48	40			
	Joanet Gros	do	25	48	8			
	Flower of Spring	England	24	42	21			
Moderately resistant.	Coeur de Boeuf de Jersey	France	25	36	32			
	Bacalan Hatif	do	26	35	15	28	29	18
	Dax	do	25	32	20			
	Sultani	Egypt	24	29	21			
	Christmas Drumhead	England	21	29	14	24	0	50
	Auvergne	France	24	25	33			
	Earliest of All	England	25	24	16	15	0	0
	Quintal	France	25	24	20			
	Amchiri	Egypt	23	22	0	20	20	10
	Habas	France	25	16	28	21	5	52
	York	do	25	4	12	21	19	14
	Fumel	do	25	12	12	24	21	29
Rather highly resistant.	Imperial	England	25	8	28	24	8	42
	Coeur de Boeuf Gros	France	25	8	8	12	8	25
	Pisé	do	25	4	28	14	0	7
	Quintal d'Alsace	do	24	4	21	25	0	12
	Bacalan Gros	do	24	4	17	23	0	9
	St. Denis	do	24	4	0	24	8	8
	Vaugirard d'Hiver	do	25	0	0	22	5	0

WILD CABBAGE

A small quantity of seed of wild cabbage was secured from Sutton & Sons, Reading, England. The first trial was made in 1924 on naturally infested soil. Of the 17 plants grown, 1 showed slight symptoms of yellows. Seed was obtained in the greenhouse the following winter from 3 of the surviving plants, each of which was self-pollinated. The strains thus secured were labeled WC–1s, WC–2s, WC–3s. Results of trials with these lots in 1926 and 1927 are given in Table 3. It is evident that most of these progenies exhibited a fair degree of resistance to yellows and that they were more resistant than the average variety of cultivated cabbage. These data should be compared with those secured from the varieties of cultivated forms.

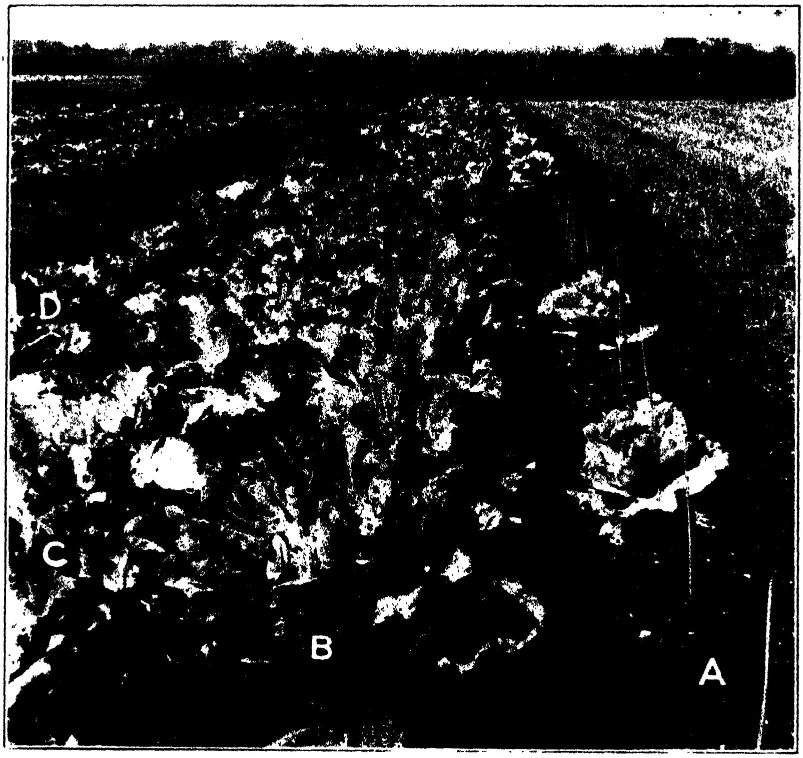

FIG. 1.—Cabbage and related forms growing on soil thoroughly infested with the yellows organism, Kenosha County, Wis. Photographed September 6, 1927. The bamboo stakes were used to mark each plant as the disease appeared. A, Danish Ballhead cabbage which showed 95 per cent total infection and 68 per cent killed or severely diseased; B, Hartman's Special cauliflower which showed 21 per cent infection but only 6 per cent dead or severely diseased; C, Large White Mammoth broccoli which showed 18 per cent infection, in all cases very slight; D, Improved Danish Brussels sprouts which showed 28 per cent infection, though only 10 per cent were severely affected. See further data in Table 3 and in text

RELATIVES OF CULTIVATED CABBAGE

Some preliminary tests were made with cabbage relatives in 1924 and 1925. Only a few varieties were used, and the data collected are not included, since they are confirmed by the more extensive trials of

1926 and 1927. It was noted during the first two seasons that cauliflower, broccoli, and Brussels sprouts were distinctly more resistant than most cabbage varieties, while kohl-rabi, collard, and kale, with the exception of Siberian kale, which was highly resistant, showed varying degrees of susceptibility. The trials of 1926 and 1927 gave opportunity for more extensive analyses. A portion of the 1927 trial plot is shown in Figure 1.

TABLE 3.—*Comparative Fusarium resistance of varieties of the cultivated and the wild forms of Brassica oleracea*

Common name	Subspecies	Variety or strain	1926 trials			1927 trials		
			Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered	Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered
				Per cent	*Per cent*		*Per cent*	*Per cent*
Cabbage	Capitata	Copenhagen Market [a]	44	73	0	149	36	52
		Hollander [a]	65	65	12	104	68	27
		Wisconsin All Seasons [b]	49	0	6	100	0	6
Wild cabbage	Sylvestris	W C-1s	23	0	26	20	25	10
		W C-2s	8	13	0	18	17	17
		W C-3s	25	4	12	60	8	7
Kale	Acephala	Tall Green Curled	21	33	67	73	27	45
		Dwarf Green Curled	25	60	40	46	67	33
		Excelsior Moss Curled	20	25	75	50	36	44
		Thousand-Headed	23	13	26	50	14	2
		Mosbach Winter	17	18	29	50	40	22
		Siberian	24	0	8	75	0	3
Collard	do	North Carolina Short Stem.	21	14	24	50	22	10
		White or Cabbage	24	13	42	49	8	6
		True Georgia	25	24	20	50	14	10
Kohl-rabi	Caulo-rapa	Short Top Early Erfurt	17	65	24	50	36	32
		Early Purple Vienna	20	40	50	50	40	16
		White Vienna	24	38	33	35	20	3
		White Goliath	21	33	52	40	15	25
Brussels sprouts	Gemmifera	Improved Danish	23	0	35	49	10	18
		Paris Market	20	0	50	50	2	18
		Dwarf Improved	18	0	28	49	2	10
		Improved Long Island	26	4	27	50	2	2
		Paris Half Dwarf	25	0	40			
		Amager Market	19	0	32			
Cauliflower	Botrytis	Hartman's Special	21	0	52	50	6	15
		Vaughan's New Snowball	23	30	30	23	4	30
		Danish Snowball	24	50	38	47	15	36
		Vaughan's Snowball	35	11	63	23	0	39
		Wieboldt's Express	23	13	43	47	6	64
		Extra Early Paris	20	5	85	25	4	76
		Half Early Paris	25	12	52	25	24	48
		Extra Early or Second Erfurt.	24	33	42	50	8	68
		Large Early Erfurt	25	16	56	23	4	61
		Le Normand's Short Stem	25	12	76	25	4	44
		Dry Weather	22	14	55	25	0	36
		Danish Perfection	22	23	45			
		Autumn Giant	21	0	0	49	2	10
Broccoli	do	Purple Cape	23	0	13	26	4	4
		Large White Mammoth	25	12	76	55	0	18
		Early Large White French	25	0	0			
		White Cape	25	4	68			

[a] Susceptible.

[b] Resistant.

The results of trials with six varieties of Brussels sprouts are given in Table 3. It should be noted that very few plants were severely affected with yellows, although an appreciable number of each variety showed slight symptoms. The damage was of minor consequence from the commercial standpoint. It is evident that any of these strains could be grown upon thoroughly infested soil with confidence in their successful evasion of the disease. This subspecies is the most uniformly resistant of any of the groups studied.

Cauliflower varieties were, as a rule, distinctly resistant to yellows, though not generally to such a high degree as Brussels sprouts. It will be seen from the data in Table 3 that in most varieties tested a comparatively large percentage of the plants showed evidence of the disease. The important distinction to be noted is that a large portion of the affected plants were only slightly attacked, and in many cases the actual damage to a variety as a whole was relatively small. (Fig. 1.) But here again, as in cabbage, varieties differ widely in their reactions. It is of interest to note particularly the wide variation among the different strains of Snowball. Undue reliance should not be placed upon a single test for a given variety, for there might exist numerous strains of that form which vary considerably in resistance. As the practical need for resistant strains of cauliflower arises, advantage should be taken of the natural yellows resistance both for its value in meeting needs temporarily and as a basis for further improvement.

Broccoli, which is very closely related to the cauliflower, probably less often encounters yellows, as it is most commonly grown as a winter crop. Curiously, however, all four varieties tested are highly resistant to *Fusarium conglutinans*, and there will probably be little need for their further improvement in this respect.

Many varieties of kale have come into use and are widely grown. Only a few of those most common in America were tested. It was found (Table 3) that the curly-leaved varieties were decidedly susceptible. The smoother leaf types such as Thousand-Headed and Mosbach Winter were on the whole more resistant, and the Siberian was very resistant. The collards are closely related to the smooth-leaved kales and are especially popular in the southern part of the United States. They were susceptible to about the same degree as Thousand-Headed and Mosbach Winter kales. Thus, as in the forms previously considered, there is considerable variation in resistance within this subspecies. The smooth-leaved forms were only moderately damaged on infested soil under conditions favorable for the disease, while curly-leaved varieties were so affected that their commercial value was seriously impaired. In any of the smooth-leaved varieties considered except Siberian there is ample opportunity to develop more highly resistant strains through selection. In the curly-leaved forms the possibility of finding resistant individuals for selection is not so certain, but in spite of the fact that all three of these varieties showed 100 per cent yellows in the 1926 trials, a few plants were resistant in two of the varieties in 1927.

Kohl-rabi appears to be the most generally susceptible subspecies with the exception of cabbage. (Table 3.) All four varieties tested were severely attacked. From their behavior it is evident that they can not be safely used on thoroughly infested soil during the season favorable for the development of yellows. Further search might possibly reveal more naturally resistant strains. On the other hand, it is evident that the way is open for improvement in resistance through selection from any of these commercial varieties by methods already shown to be successful with cabbage.

DISCUSSION

Brassica oleracea is interesting for study of the variation in resistance to *Fusarium conglutinans* among its cultivated subspecies and varieties. Whether or not the present wild form of Europe is the progenitor of our cultivated forms or whether it also has evolved from some common ancestor is problematical. The wild form of cabbage as we now know it, when tested on soil infested with *F. conglutinans*, shows a high degree of resistance, but a study of the progeny of individual plants shows them to be segregating in this character. In fact, of 154 plants tested from three selfed progenies, 33 became diseased, showing a segregation reasonably close to 3 resistant to 1 susceptible. This is in accordance with previous findings in the case of cultivated cabbage, where resistance behaves as a single dominant Mendelian character.[10]

The apparent absence of the Fusarium disease in Europe, even in regions climatically favorable for its development, seems to substantiate the belief that the parasite is of American origin. The development of the subspecies of *Brassica oleracea* now known and their further segregation into numerous varieties under cultivation have thus gone on out of contact with this disease. If the yellows organism had been present and active it is not unlikely that natural elimination of susceptible individuals of this species, which normally is cross-pollinated, would by this time have resulted in quite general resistance. On the contrary, the state of affairs that did actually exist would naturally lead to no uniformity in resistance. Chance might result in certain varieties becoming highly resistant and in others becoming very susceptible.

The data that have been presented show this to be the case. The cabbage varieties range from the highly resistant types such as St. Denis and Vaugirard d'Hiver to the very susceptible forms such as Danish Ballhead. In kale the very resistant Siberian stands at one extreme and the susceptible moss-curled types at the other. All Brussels sprouts varieties tested are highly resistant, while all kohl-rabi forms examined are very susceptible. Out of this whole series, however, the important fact is revealed that no variety tested, with the possible exception of one of the moss-curled kales, appeared to be homozygous in the susceptible character. Thus since resistant individuals occur the opportunity exists to improve by selection any given variety in this respect, as has been already amply demonstrated with cabbage.

The observations here reported are not intended as a final statement of the comparative behavior of the subspecies and varieties mentioned. Since the tests were made in a single locality, the results must be taken only as an indication of the relative resistance of the varieties used. However, experience with resistant strains of cabbage has led to the conclusion that the tests thus employed give a fair index to behavior to be expected in other localities, granted the general assumption that the rise in the average temperature may be expected to increase the incidence of yellows. Variation with locality in the pathogene leading to selective pathogenicity has not yet come to the attention of the writers, but should be constantly watched for as a possible complicating factor.

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SUMMARY

A survey was made of the behavior of wild cabbage and a number of varieties of the cultivated subspecies of *Brassica oleracea* when grown upon soil infested with *Fusarium conglutinans*.

Although most cabbage varieties commonly used in America are very susceptible to yellows, various degrees of resistance were found when a number of European varieties not ordinarily used in America were tested.

The wild cabbage of Europe was highly resistant, but selfed progenies from individual plants showed about one-fourth of the plants diseased.

Brussels sprouts and broccoli varieties, though showing a considerable number of plants slightly affected, were not seriously damaged by yellows.

Cauliflower varieties varied somewhat in reaction, but in general they were damaged to a greater degree than broccoli or Brussels sprouts.

The kale varieties differed widely in susceptibility. The Siberian kale was very resistant, while the curled-leaf types were very susceptible. The smooth-leaf varieties and the collards occupied an intermediate position.

The kohl-rabi varieties were all very susceptible.

Within all of the forms tested a sufficient number of individuals survived to make it possible to improve their resistance through selection.

THE ISOLATION OF THE FUNGUS THAT CAUSES CITRUS MELANOSE AND THE PATHOLOGICAL ANATOMY OF THE HOST[1]

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INTRODUCTION

Citrus melanose[4] is characterized by the presence of small, pustular lesions that look like drops of caramelized sugar on leaves, twigs, and fruits. These lesions are initiated while the parts are still young, and at maturity they may occur as isolated dots or may be arranged in streaks, rings, or extensive irregular patches. The fungus that causes melanose possesses two stages, a pycnidial one, *Phomopsis citri* (*2*),[5] described in 1912, and an ascigerous one, *Diaporthe citri* (*10*), described in 1926.

Melanose was first described in 1896 by Swingle and Webber (*8*), who first observed it in November, 1892, at Citra, Fla. They were unable to establish the cause definitely, but assumed it to be a fungus. Furthermore, until now, no one has succeeded, even after repeated attempts, in isolating the causal organism from melanose lesions. The pathogene has been isolated repeatedly, however, from dead twigs and from fruits affected with stem-end rot, two manifestations of the disease that can not properly be designated melanose, but that result from infection by the same organism. The relationship between stem-end rot and dying back of twigs has been determined by the investigations of Fawcett (*2*) and the relationship of melanose to the other two manifestations by Stevens (*7*) and by Floyd and Stevens (*4*).

Fawcett (*2*) isolated *Phomopsis citri* from the interior of fruits affected with stem-end decay and from the interior of dead twigs. He showed by the cultural similarity of isolations from these two sources and by inoculations that these two forms of the disease are caused by the same fungus.

Floyd and Stevens (*4*), who did not suspect at first that melanose and stem-end rot were related, later found that the two forms were undoubtedly caused by the same fungus. They occasionally found particles of fungous hyphae in melanose lesions, but were not able to demonstrate whether these were parts of the causal organism or of some secondary invader. They concluded from their microscopic examination of lesions of different ages that no bacterial or fungous organism that could be considered a cause of the disease could be found connected with the affected tissues or the adjoining cells. They tried to isolate the causal organism by planting on various media bits of

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[4] This usage of the term "melanose" is in accord with that first employed by Swingle and Webber (*8*) and is employed in the same restricted sense throughout this paper.

[5] Reference is made by number (italic) to "Literature cited," p. 252.

diseased leaves that had been soaked in 1:1,000 mercuric chloride for three to five minutes and washed in sterile water. In nearly every case Colletotrichum overran the cultures and made it impossible to isolate any other fungus that might have been present.

Stevens (*7*) in 1918 concluded that there was no growth of the fungus within the affected tissues. All attempts to isolate the fungus from artificial inoculations gave negative results, and the organism was never obtained from spots or markings formed naturally. Melanose infections were secured, however, from inoculations with pure cultures of *Diaporthe citri.*

Winston, Bowman, and Bach (*9*) made fully 1,000 systematic attempts to recover the causal organism from melanose blemishes, but without success in a single instance. Leaf and fruit tissues from both old rough lesions and young, almost invisible spots were cultured. Such surface disinfecting agents as ethyl alcohol, ether, acetic acid, mercuric chloride, and hydrogen peroxide were used, after which the material was rinsed in sterile tap water before being planted in cultures. Repeated attempts were also made to isolate the organism without first subjecting the lesions to surface disinfection.

While the results of previous investigations of melanose, stem-end decay, and the dying back of twigs and branches have left no reasonable doubt as to the identity of the fungus that causes them, the failure to isolate the pathogene from melanose lesions has made it impossible to fulfill completely Koch's postulates. The present study is, therefore, concerned both with the isolation of the pathogene from melanose lesions and with the pathological anatomy of the host, to which no special attention has been given except by Floyd and Stevens (*4*). The results show that it is possible to complete the rules of proof of pathogenicity of the fungus that causes melanose, and the findings and interpretations relative to anatomical changes that are herein recorded are believed to contribute further to an adequate understanding of the disease.

ISOLATION

Young artificially inoculated orange leaves were used in the preliminary trials. These leaves had been inoculated eight days previously with suspensions of conidia from pure cultures. The young lesions, which were plainly visible at this time, were excised, dipped in 95 per cent alcohol, flamed, and placed on slants of 2 per cent potato-dextrose agar. The resultant mycelial growth in 7 of the 10 slants presented the characteristic appearance of *Diaporthe citri,* while no growth occurred in the other three tubes. This was so unusual in the light of previous experience that subcultures were made on sterilized stems of pigeon pea (*Cajanus indicus*), a substratum favorable for pycnidial formation, in order to induce the development of fruiting bodies. Conidia that were typical of the pycnidial stage, *Phomopsis citri,* were produced in due time in these subcultures. Additional proof of this identity was secured by the inoculation of young grapefruit leaves with these cultures. Infection resulted and typical melanose markings developed.

As a consequence of the successful isolation of the pathogene in this preliminary trial, further attempts were made to obtain the melanose fungus in culture from lesions of varying ages on leaves, twigs, and fruits. In all cases the surfaces of the affected parts were disinfected by dipping them in 95 per cent alcohol and removing the alcohol by flaming. The lesions were then excised and placed on agar plates or slants. As soon as growth had proceeded sufficiently, which usually required four or five days, subcultures were made from portions of the colonies that looked like the melanose fungus. In a number of cases, especially when young lesions were used, the fungus appeared in pure culture in the planted plates or tubes and it was not necessary to make subcultures. As a routine practice, however, the fungus can not be isolated without making subcultures so as to separate the pathogene from the various secondary invaders that early overgrow the cultures. It is possible that the failure of other investigators to isolate the melanose organism is due in part at least to their failure to use subcultures, and in consequence the causal fungus was crowded out or intermingled with those fungi that grew more rapidly.

In order to test the efficacy of this method of surface disinfection, two normal grapefruit leaves were immersed on June 29, 1926, in a suspension of conidia of the Phomopsis stage. After they had dried one was dipped in alcohol, flamed, and bits of the leaf tissue were planted on agar. Fragments of tissue from the other were planted without disinfection. Of six plantings made from each leaf no growth appeared in any from the first leaf, while three cultures of the melanose organism and three of other species of fungi were obtained from the second. This test was repeated on July 12, 1926, when six plantings were again made from a disinfected leaf and six from one that was not given surface disinfection. Again no growth appeared in the first case, and the second yielded two cultures of *Diaporthe citri* and four of other organisms. A third trial made in the same manner on March 18 employed 20 plantings of leaves that were given surface disinfection and an equal number that were not disinfected. No fungous growth appeared around any of the plantings from disinfected leaves, whereas 14 cultures of the melanose fungus were recovered from those that were not disinfected, 5 yielded other fungi, and the other remained sterile. This method of surface disinfection is therefore regarded as effective, at least for comparatively normal leaf tissue. That it is also highly effective for tissues with minute fissures is indicated by the results given later. (Table 3.)

During the course of this investigation several additional tests of the effectiveness of this method of surface disinfection were made, employing leaves and twigs that were free from melanose lesions and apparently normal but on the surface of which the conidia may reasonably be presumed to have been present. The results are summarized in Table 1.

No cultures of the melanose organism were secured in this series of 108 trials, although 4 yielded *Colletotrichum gloeosporioides*, a fungus that appears always to be present on citrus throughout Florida. It seems improbable that the conidia of this Colletotrichum would survive disinfection and at the same time those of the melanose fungus be destroyed. It may be that the tissues were only apparently normal and that the fungus had established itself in minute fissures.

TABLE 1.—*Results of experiments to isolate Diaporthe citri from apparently normal tissues*

Source of material	Date of experiment	Number of plantings made	Number of plantings yielding—		Number of plantings remaining sterile
			Diaporthe citri	Miscellaneous organisms only	
Old orange leaves	Oct. 31, 1925	9	0	0	9
Mature grapefruit leaves	do	6	0	0	6
Leaves of Chaetospermum glutinosum	Nov. 7, 1925	6	0	0	6
Mature orange leaves	do	14	0	1	13
Leaf scars on orange twigs	Nov. 15, 1925	15	0	0	15
Leaves of oranges from June flush	June 15, 1926	16	0	1	15
Orange twigs of spring growth	June 19, 1926	12	0	0	12
Old grapefruit leaves	July 12, 1926	11	0	0	11
Mature grapefruit leaves	June 15, 1927	19	0	2	17
Total		108	0	[a] 4	104

[a] Colletotrichum gloeosporioides.

The attempts to isolate *Diaporthe citri* from melanose lesions have extended over a period of three seasons. Use has been made of lesions on leaves, twigs, and fruits of orange and grapefruit, on leaves of tabog (*Chaetospermum glutinosum*), on twigs of Mexican lime, and on fruits of faustrime (Mexican lime × Australian finger lime). Table 2 contains a summary of the essential features and results of these isolation experiments.

A total of 115 cultures of the melanose fungus have been obtained from 506 plantings of melanose lesions. These lesions varied in age from 6 days to approximately 9 months. Those of definitely known age were obtained by artificial inoculation or occurred following rains on April 8, 1926, and February 14, 1927. The age of the other natural infections was estimated from the age of the flush of growth on which they occurred.

It is of special interest to note, too, that cultures from lesions that had not yet advanced to the stage in which the cuticle had become fissured yielded the pathogene in pure culture or else remained sterile. In general, a larger proportion of successful attempts resulted from isolations from young lesions than from old ones, since various other fungi, primarily *Colletotrichum gloeosporioides* and secondarily *Diplodia natalensis*, were always present in mature lesions. In no case was the percentage of successful attempts to isolate *D. citri* from melanose lesions as large as usually results from attempts to isolate other plant pathogenes from other host tissues. One probable reason for the relatively small number of successful attempts to isolate the melanose fungus is, as will be shown subsequently, that the infected tissues are flooded with gum, which may envelop the mycelium and prevent it from growing out of the tissues in culture. Furthermore, as will be shown in this paper, anatomical studies reveal the fact that the host cells are disintegrated by enzymes. A concomitant digestion of the hyphae of the pathogene may therefore be expected.

Table 2.—*Results of experiments to isolate Diaporthe citri from melanose lesions*

Approximate age of lesions	Source of material	Type of infection	Date of experiment	Number of plantings made	Number of plantings yielding—		Number of plantings remaining sterile
					Diaporthe citri	Miscellaneous organisms only	
6 days	Young grapefruit leaves	Artificial	May 23, 1926	6	2	0	4
Do.	do.	do	June 15, 1926	15	4	2	9
8 days	do.	do.	Oct. 17, 1925	10	7	0	3
10 days	Grapefruit leaves	Natural	Feb. 24, 1927	24	5	7	12
15 days	Orange twigs	Artificial	July 12, 1926	8	1	0	7
Do.	Grapefruit leaves	do	July 27, 1926	11	4	3	4
21 days	Lime twigs	Natural	June 30, 1927	19	4	0	15
27 days	Young orange fruit	do.	May 5, 1926	6	2	1	3
34 days	Lime twigs	do.	July 11, 1927	16	8	8	0
Do.	Young grapefruit fruit	do.	May 12, 1926	15	6	5	4
42 days	Grapefruit leaves	do	July 12, 1926	15	2	1	12
Do.	Orange leaves	do.	July 16, 1926	12	1	11	0
Do.	Leaves of Chaetospermum glutinosum.	do.	Oct. 20, 1925	10	2	6	2
44 days	Grapefruit leaves	do.	July 22, 1927	23	4	4	15
45 days	Orange fruit	do	May 12, 1926	20	5	11	4
49 days	Grapefruit leaves	do.	July 27, 1926	7	1	0	6
63 days	do.	do.	June 11, 1926	8	2	3	3
Do.	Orange fruit	do.	do.	3	2	0	1
Do.	Grapefruit twigs	do.	do.	6	1	0	5
70 days	Grapefruit fruit	do.	Aug. 12, 1926	11	1	10	0
71 days	Orange leaves	do.	June 19, 1926	12	3	6	3
80 days	Orange fruit	do.	June 28, 1926	6	1	0	5
Do.	Grapefruit leaves	do.	do.	10	4	2	4
84 days	Orange leaves	do.	Dec. 17, 1925	6	2	1	3
87 days	Grapefruit leaves	do.	June 15, 1927	23	4	1	18
91 days	Orange leaves	do.	Sept. 11, 1926	20	7	2	11
94 days	Grapefruit leaves	do.	June 22, 1927	22	4	5	13
Do.	do.	do	July 12, 1926	24	5	7	12
Do.	Grapefruit twigs	do.	do.	9	1	8	0
Do.	Faustrime fruit	do.	July 16, 1926	8	1	4	3
103 days	Orange leaves	do.	Dec. 11, 1925	6	2	1	3
130 days	Grapefruit fruit	do.	Aug. 16, 1926	12	1	11	0
131 days	Orange fruit	do.	Aug. 17, 1926	27	3	16	8
149 days	Faustrime fruit	do.	Sept. 4, 1926	11	1	5	5
152 days	Orange fruit	do.	Sept. 7, 1926	6	0	4	2
167 days	Grapefruit leaves	do.	Sept. 22, 1926	8	1	7	0
179 days	do.	do.	Oct. 4, 1926	18	2	14	2
183 days	do.	do.	Oct. 8, 1926	10	1	1	8
270 days	do.	do	June 11, 1926	13	7	1	5
278 days	do.	do.	June 19, 1926	10	1	0	9
Total				506	115	168	223

The reisolation of the melanose fungus from infections resulting from artificial inoculation makes it possible to complete, for the first time, Koch's rules of proof of pathogenicity.

Although young lesions yielded the melanose pathogene in pure culture, and its presence in mature lesions is presumably due to the fact that the mycelium remains alive from the time of primary infection, this may not necessarily be the case. Additional evidence on this point comes from the planting of lesions resulting from mechanical injuries and from attacks of the rust mite (*Phyllocoptes oleivorus* Ashm.). The data on these tissue plantings are assembled in Table 3.

TABLE 3.—*Results of experiments to isolate Diaporthe citri from various lesions on grapefruit other than melanose*

Source of material	Date of experiment	Number of plantings made	Number of plantings yielding		Number of plantings remaining sterile
			Diaporthe citri	Miscellaneous organisms only	
Abrasions from rubbing on fruit	May 12, 1926	10	0	0	10
Greasy melanose on leaves of September, 1925	June 11, 1926	10	1	4	5
Abrasions on fruit from contact with limbs	June 15, 1927	25	0	19	6
Russeted fruit from rustmite injury	do	20	1	16	3
Russeted fruit	June 22, 1927	21	0	2	19
Abrasions on fruit from rubbing	do	22	0	2	20
Abrasions on fruit	June 30, 1927	24	1	9	14
Abrasions on June bloom fruit	July 11, 1927	12	0	12	0
Rust-mite russeted fruit	do	12	0	10	2
Russeted fruit	July 22, 1927	21	1	12	8
Total		177	4	86	87

Four of the 177 trials in this series yielded cultures of the melanose fungus, and *Colletotrichum gloeosporioides* again predominated among the miscellaneous organisms present. In the light of the previously mentioned results on the effectiveness of the method of surface disinfection, it seems improbable that these cultures originated from conidia that were present on the surface or were lodged within crevices of the lesions. As is well known, not only Colletotrichum but also the melanose fungus can occupy tissues saprophytically. The dead-twig manifestation of the disease, in the case of the latter organism, is evidence of this condition. It seems more reasonable, therefore, to believe that the colonies resulted from mycelia within the tissues. This is substantiated by microscopic examination of russeted and abraded citrus tissues, which reveal the universal presence of hyphae within them.

PATHOLOGICAL ANATOMY

Collections of material from natural infections that occurred during April and June were used in studying the anatomy of lesions in advanced stages of development. Infections resulting from artificial inoculations with pure cultures were employed in studying the early stages of the disease. The inoculations were made by wetting bits of absorbent cotton in suspensions of conidia and placing them upon tender leaves and fruits of grapefruit. These were then protected against desiccation by being wrapped in waxed paper. Both free-hand and paraffin sections stained in Haidenhain's iron-alum haematoxylin were used. Infection is effected 36 to 48 hours after inoculation by direct penetration of the upper epidermis. This phenomenon can best be observed in free-hand sections cut parallel to the leaf surface. The germ tube penetrates the cuticle and passes downward between the lateral walls of adjacent epidermal cells. (Fig. 1, A.) Thence it branches and extends intercellularly between the palisade parenchyma.

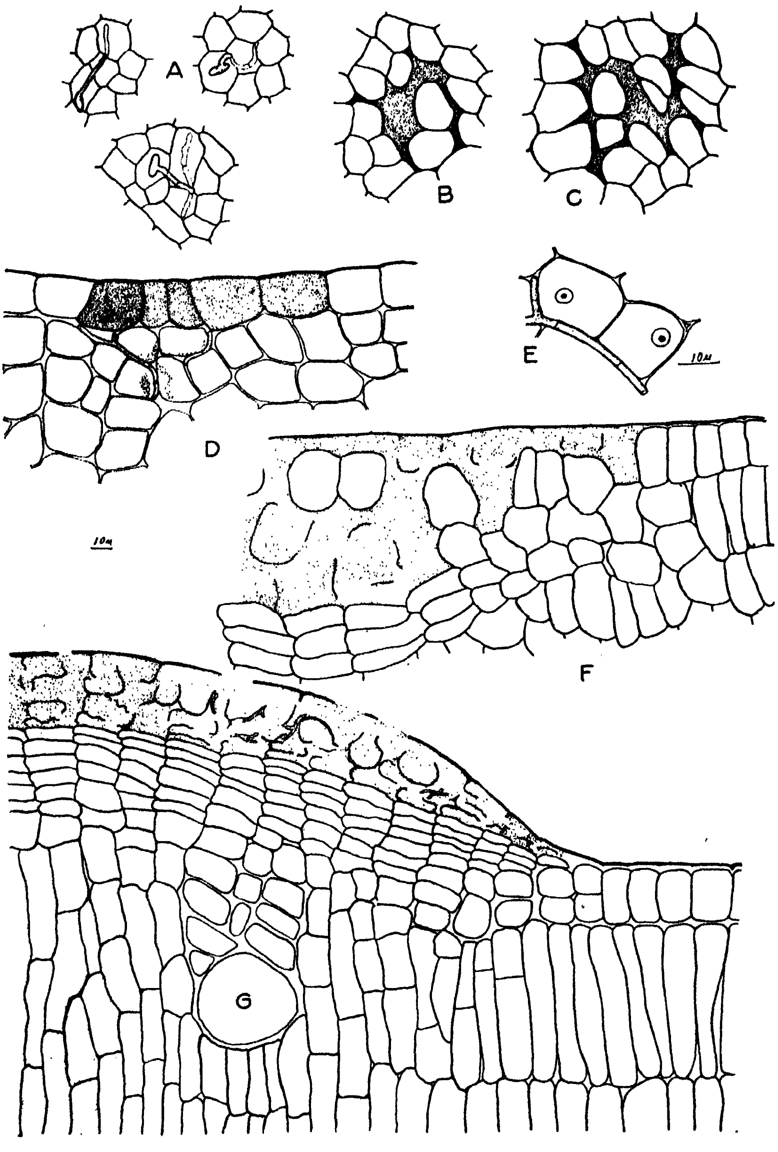

FIG. 1.—A, surface views of grapefruit leaves showing penetration of conidial germ tubes into vertical walls of epidermal cells, 36 hours after inoculation. B, surface view of a grapefruit leaf bearing a melanose lesion 80 hours old, showing gelatinization of cell walls. C, lesions on grapefruit leaves 110 hours old, with the dissolution of primary cell membranes, and the resultant formation of free-floating cells. D, margin of a melanose lesion 80 hours old on young fruit in vertical section. The center of the lesion is sunken, the cells are collapsed or filled with gum, and the hyphae are intercellular. E, intercellular hypha of *Diaporthe citri*. F, vertical section of a lesion 7 days old showing dissolution of cell walls with resultant gummosis and the beginning of the formation of the suberized layer. G, mature melanose lesion in which the corky layer has completely separated the affected tissues from the subjacent normal tissues. The cuticle has been ruptured by tensions and the gum mass has become brown. (A, B, C, D, F, and G were drawn to the scale shown below D, and E to the scale at its right)

There is no evidence of infection visible to the unaided eye until the fourth day after inoculation. At this time the epidermal cells and intercellular spaces are filled with a gummous substance that gives a bright-red precipitate when treated with hydrochloric acid and phloroglucin. This gummous substance is manifestly a hemicellulose derivative resulting from the digestion of the cell wall by pectic enzymes. That such enzymes are present is shown by cultures of the melanose fungus on pectin agar, which was made by the addition of pectin to plain agar. Pectin from two sources was used, a commercial lemon pectin in powdered form and a commercial apple pectin purified by repeated precipitation with alcohol. The initial reaction of the media was adjusted to approximately pH 5, and methyl red was added as an indicator. The color disappeared in a broad area surrounding the colonies, which indicated an increase in alkalinity as a result of the growth of the fungus. A narrow clear zone formed at the borders of the colonies resulted from the digestion of the pectin by enzymes.

It is apparent from examination of lesions four to five days after inoculation that the primary cell membranes are involved in gummous degeneration and that the accumulation of gum between cells forces them apart. Thin-walled cells floating free in the gum mass can be seen at this stage. (Fig. 1, B and C.) The degeneration of the inner lamellae of the walls of these free-floating cells proceeds centripetally until the cell contents are freed and become mixed with the gum matrix. The dissolution and collapse of cells results in the formation of a depression, which marks the site of the lesion. The cuticle, however, remains intact. This is shown by the microscopic appearance of both free-hand and paraffin sections. (Fig. 1, D.) It is shown indirectly by the absence of secondary invaders from isolations from lesions 6 to 7 days old, whose surfaces have been disinfected, since such isolations either have yielded the melanose fungus alone or have remained sterile.

By the time the lesions are 7 days old the differentiation of a phellogen layer has begun in an area several cell layers in advance of gummosis. This is manifest by the formation of cell walls in a plane parallel to the leaf surface. (Fig. 1, F.) The epidermal cells and any of the subepidermal tissues may be involved in the formation of the suberized layer. As a result, a saucer-shaped suberized tissue which completely separates the invaded normal tissues, is formed between them. The growth of this corky tissue proceeds until the tiers of cork are 7 to 12 cell layers in thickness. Meanwhile the normal growth of the healthy tissues beneath the lesions results in everting the corky layer and thus in raising the lesion so that it protrudes about the surface. (Fig. 1, G.) At this stage when the lesions are abundant the affected parts are rough to the touch like sandpaper.

Coincident with the development of the corky layer the tensions on the cuticle result in its rupture and the gum mass on exposure to the air becomes brown and dry. This permits various fungi to penetrate through the fissures. The necrotic tissues of old lesions yield in culture not only the melanose fungus but also species of Colletotrichum, Gloeosporium, Fusarium, Pestalozzia, Cladosporium, and Alternaria.

DISCUSSION

The foregoing observations on penetration and on the presence of the melanose pathogene within the tissues are at variance with previously published accounts. Floyd and Stevens (*4*) stated that there was apparently no vegetative growth of the fungus within infected tissue in causing the formation of melanose lesions, as evidenced by the fact that stained sections failed to reveal mycelium either within diseased tissues or within adjoining cells. They suggested that the lesions may possibly be caused by some chemical substance or toxic principle that is eliminated by the germination or death of the conidia. In support of this view Stevens (*7*) reported that dilute lemon juice, when sprayed on young foliage, caused the formation of markings quite typical of melanose. Whatever may be the action of chemicals, the present observations show that the lesions are initiated by the direct penetration of the tissues by the melanose fungus, which accords with infection phenomena in general. However, the observations on the relative abundance of mycelia within melanose lesions, when comparison is made with lesions produced by other pathogenes on other hosts, lead to the conclusion that the hyphae of *Diaporthe citri* are very scarce even in young lesions. The occasional isolation of this fungus from lesions other than melanose markings can most reasonably be interpreted as showing that it may occupy such tissues as a secondary invader.

Microscopic examination discloses the fact that in the formation of melanose lesions the zone of gummous degeneration extends in advance of the mycelium, which shows that this gum is undoubtedly the result of enzymotic action. This observation accords with the well-established fact that the freshly exuded gum in woody plants contains a pectin-dissolving enzyme (*5*) and that the production of gum is due to enzymotic action. While gum formation in citrus [6] may occur as a response to injury from any cause, as evidenced by its occurrence in connection with such diseases as exanthema, psorosis, and foot rot, the proximate cause in the case of melanose is the pathogene itself through its ability to secrete pectic enzymes.

It is not necessary to assume that the dissolution of the cell walls is due entirely to enzymes secreted by the fungus, since Hodgson (*6*) has shown in his studies on abscission of leaves and fruits that the pectic enzymes or their appropriate zymogens exist normally within the tissues of citrus.

The manner of the formation of the corky layer presents no novel features, but appears to correspond in all essentials with cork formation of other plants. This layer is therefore to be regarded as wound cork, which is well known to arise as the normal response to traumata.

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[6] The monograph by Butler (*1*), to which the reader is referred for a comprehensive account of gummosis, contains a review of the numerous investigations on this problem. The more recent studies by Fawcett (*3*) further contribute to an understanding of this phenomenon.

SUMMARY

Investigators have hitherto been unsuccessful in isolating *Diaporthe citri* from citrus melanose. During the present study 115 isolations have been secured from a total of 506 trials. Isolations have been made from leaves, twigs, and fruits from lesions that varied in age from 6 days to approximately 9 months. The isolation of *D. citri* from melanose lesions has made it possible to complete Koch's rules of proof of pathogenicity.

Surface disinfection was accomplished by immersion in alcohol and removal of the alcohol by flaming. The lesions were then planted, and as soon as the pathogene had grown from them it was separated from the secondary invaders by means of subcultures.

Direct penetration of conidial germ tubes has been observed. The mycelium is intercellular, and the tissues are disintegrated in advance of the hyphae.

Two phenomena occur in the formation of melanose lesions—gummosis and suberization. Gummosis is the result of enzymotic action, primarily of pectic enzymes, and the melanose fungus itself is able to secrete these enzymes. Suberization is a wound response of common occurrence in citrus and in many other plants.

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THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION[1]

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INTRODUCTION

From the beginning of the energy-metabolism studies at this college, in 1901–2, under the direction of Armsby, a continuing problem, of obvious significance, has been the effect of the plane of nutrition on the several factors of energy loss and expense in the utilization of food, which, collectively, subtracted from the gross energy of the food, leave the net energy available to the animal for purposes of maintenance and production.

The evolution of Armsby's ideas on this subject has been traced in a recent paper by Forbes, Kriss, and Braman (*11*).[3]

In a number of recent papers from this institute, but especially in the one to which the authors have just referred, evidence has been presented, from metabolism experiments with cattle, for the belief that the heat production—a prominent factor in the determination of net-energy values—is not a rectilinear function of the quantity of the feed. This idea has been expressed in the following language (*11, p. 170*):

> On account of the great variability of computed maintenance values and the fact that the computed maintenance from supermaintenance periods is always a materially lower value than is the directly determined fasting katabolism . . . the writers believe that the heat increment does not thus vary directly as the feed.

The same principle is also implied in the determination of different rates of utilization of feed energy for maintenance, body increase, and milk production, as reported in an earlier paper by Forbes, Fries, Braman, and Kriss (*9*).

The effect of this observation, therefore, has been to throw open the whole problem of the subject of this paper, especially as it has to do with the determination of net-energy values of feeds.

A conception fundamental to the above expressions regarding net-energy values and maintenance requirements is the assumption that the maintenance quota of net energy is the same at all planes of nutrition.

Since the fasting katabolism is the accepted measure of this quota, and since, obviously, this value can be determined only during actual fast, its constancy at all planes of nutrition rests on assumption, or definition.

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[2] The authors take this occasion to express their grateful indebtedness to the members of the Department of Animal Husbandry of this college for many courtesies and for valued cooperation in connection with this research, especially to Prof. F. L. Bentley for the provision of the steers used as subjects, to Dr. J. F. Shigley for veterinary advice and service, and to Asst. Prof. P. T. Ziegler for cooperation in obtaining slaughter data in the study of fast.

[3] Reference is made by number (italic) to "Literature cited," p. 299.

It is impossible, therefore, to separate the maintenance requirement of net energy from the remainder of the heat production (the heat increment) in a critically scientific manner; but it is necessary, nevertheless, that we have some one definite value, conventional if not absolute, for the maintenance requirement of net energy at all planes of nutrition, in order to be able to compute the feed requirements of animals for both maintenance and production, as is necessary for guidance in feeding practice.

The above assumption is typical of a number of such postulates, impossible of proof, and warranted in part by definition, convention, or usefulness, which are necessary to the derivation of any system of values of feeds based upon Armsby's net-energy conception.

Care is necessary, therefore, on the part of the student, not to be confused by the two scientific attitudes expressed in the determination of a net-energy value, based as it is in part on the most refined and particular of animal experimentation, but in part also on arbitrary procedures adopted only as means for the establishment of practical measures and guides.

However great the difficulties and compromises involved in the determination of net-energy values of feeds, the principle of this estimation is exceedingly simple, and certainly correct. The total of the expenses and losses of food utilization, in terms of energy, subtracted from the gross energy of the food, yields the net energy available for maintenance and production; and these expenses and losses are (1) the potential energy of the visible excreta, and of the methane produced by carbohydrate fermentation, and (2) the heat increment—this latter comprising not only all direct expenditures of energy in prehension, mastication, deglutition, fermentation, rumination, peristalsis, digestion, transportation, anabolism, dynamic stimulation, and excretion; but also any indirect increase in heat production, either through voluntary or involuntary activity, *which has resulted from the consumption of feed* (except, in the practice of this institute, that the effects of feed on the activity of the animal may be modified by the computation of the heat production to standard conditions as to time spent in the standing and the lying positions).

The inclusion of the last factor as a part of the heat increment is necessitated by the assumption that the fasting katabolism as determined after a period of maintenance feeding is the measure of the maintenance quota of net energy at all levels of nutrition—because, since the maintenance quota is assumed to be constant, all observed increases of heat production following and due to feed consumption must be considered as a part of the heat increment.

Apparently any possible determinations of net-energy values of feeds must involve certain conventional procedures and general standardization of methods. Such data, therefore, are not absolute, but seem to the writers to be the most significant and useful measures that have been proposed, for purposes of practical guidance, in a complex physiological situation in which perfect order, of the sort implied by these values, does not exist.

PLAN OF EXPERIMENTATION

A series of metabolism experiments was conducted, primarily for the purpose of studying the energy metabolism as related to the plane of nutrition, but also permitting observations on several important associated problems.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals*

Period No.	Steer No.	Preliminary feeding period on experimental rations	Total digestion period	Calorimeter period	Plane of nutrition	Rations fed daily				Average live weight of animals
						Roughage		Concentrate		
						Kind	Amount	Kind	Amount	
							Kgm.		*Kgm.*	*Kgm.*
1	47	Nov. 24–29	Nov. 30–Dec. 17	Dec. 14–17	Twice maintenance	Alfalfa hay	4. 29	Corn meal	4. 29	486. 2
2	36	Dec. 8–13	Dec. 14–31	Dec. 28–31	do	do	4. 048	do	4. 088	482. 9
3	47	Dec. 21–27	Dec. 28–Jan. 14	Jan. 11–14	Half more than maintenance	do	3. 250	do	3. 250	494. 6
4	36	Jan. 1–10	Jan. 11–28	Jan. 25–28	do	do	3. 090	do	3. 090	490. 2
5	47	Jan. 14–24	Jan. 25–Feb. 5	Feb. 2–5	Half less than maintenance	do	1. 073	do	1. 083	474. 8
6	36	Jan. 29–Feb. 6	Feb. 7–19	Feb. 16–19	do	do	1. 073	do	1. 088	471. 2
7	47	Feb. 5–14	Feb. 15–Mar. 4	Mar. 1–4	Maintenance (mixed ration)	do	2. 145	do	2. 188	484. 8
8	36	Feb. 21–28	Mar. 1–18	Mar. 15–18	do	do	2. 155	do	2. 181	481. 2
9	47	Mar. 11–14	Mar. 15–Apr. 1	Mar. 29–31	Maintenance (hay alone)	do	6. 50	do		499. 0
10	36	Mar. 23–28	Mar. 29–Apr. 15	Apr. 12–15	do	do	6. 50	do		499. 9
11	47			Apr. 19–23 [a]	Fast					
12	36			May 3–7 [b]	do					

[a] Days 4, 5, and 6 of fast.

[b] Days 5, 6, and 7 of fast.

The schedule of experimentation comprises Table 1. These experiments, 12 in number, consisted of a duplicate series, with two 2-year-old steers, at 5 planes of nutrition—1 at fast, 1 each at 3 other planes of nutrition, and 2 at the remaining plane, which was maintenance.

The rations were composed of corn meal and alfalfa hay, in equal weights of dry matter, at four planes of nutrition other than fast, and a second ration of alfalfa hay alone at the maintenance level.

This outline of experiments, in tabular form, is presented below:

Planes of nutrition studied	Order of treatments in experimental program	Rations fed
Twice the maintenance requirement	I	Corn meal; alfalfa hay, 1 : 1
Half more than the maintenance requirement	II	Corn meal; alfalfa hay, 1 : 1
Maintenance (energy equilibrium)	IV and V	a. Corn meal; alfalfa hay, 1 : 1 b. Alfalfa hay alone.
Half of the maintenance requirement	III	Corn meal; alfalfa hay, 1 : 1
Fasting	VI	None.

The experiments were of the kind which is standard in the energy metabolism studies with cattle at this institute, a unit ordinarily consisting of a 28-day interval, embracing a 10-day preliminary period on the experimental feeding treatment which is to follow, and an 18-day period during which the visible excreta are quantitatively accounted for, the last three days of the 18 also constituting a continuous respiration-calorimetric period, during which the heat produced and the gaseous metabolism are measured.

The digestion periods were conducted at the times indicated by the second column of dates, and were of the above-mentioned standard 18-day length, except as necessarily altered on account of irregularity of behavior of the steers.

The transition feeding periods, in the course of which the steers were changed from one plane of nutrition to another, were commonly 10 days in length; and the portions of these 10-day intervals during which the steers received exactly the quantities of feed to be given during the digestion periods to follow were as indicated in the first column of dates.

The calorimeter periods, other than those during fast, Nos. 1 to 10, were each, as usual, three days in length, barring one incomplete day in period 9, and each was preceded by a 14-hour preliminary interval during which the calorimeter was got into balance, with the animal inside, and all accessory equipment was established in regulated operation.

The calorimeter periods during fast (Nos. 11 and 12) were four days in length, in addition to the usual 14-hour preliminary period.

The length of this preliminary period was determined in part each by a desire to provide for all ordinary requirements, with a liberal excess as a margin of safety, and by considerations of convenience.

Each calorimeter period began and ended at 6 a. m., and therefore covered 18 hours of the first calendar day, and 6 hours of the last such day, of each experimental interval.

Of each of the two fasting periods (Nos. 11 and 12) the heat production of the last three days only was used as a measure of the maintenance requirement of net energy. These were the fourth, fifth, and sixth days of fast in the case of steer No. 47, and the fifth, sixth, and seventh days of fast in the case of steer No. 36.

Referring to the above tabular outline of experiments—the quantities of feed given during periods 1 and 2—about twice those required to keep the animals in energy equilibrium—were, for these steers, approximately full feed. They would not regularly consume more without leaving a part.

In periods 3 and 4 the steers received half more than a maintenance ration; in periods 5 and 6, half of the maintenance; in periods 7 and 8, maintenance; and in periods 9 and 10, maintenance again, but with a ration of alfalfa hay alone instead of the mixed ration of grain and hay.

The purpose of the periods on hay alone was to make possible the determination of the net-energy values of the individual feeds used—the alfalfa directly, and the corn by difference.

METHODS OF EXPERIMENTATION AND COMPUTATION

The methods employed in this study were in general the same as those described in the recent published work of this institute, but several new procedures were introduced. These were (1) the adoption of the area of the removed hide as the measure of the surface area of the animal; (2) the use of the respiratory quotient and (3) of the amount of the feed residues in the alimentary tract, as well as the heat production, as usual, as criteria in the standardization of conditions for the determination of the fasting katabolism as the measure of the maintenance quota of net energy, (4) the correction of the heat production to correspond to a uniform live weight and maintenance requirement of net energy, in the comparison of the heat production of an animal at different planes of nutrition, (5) the use of a new method for computing net-energy values of individual feeds for the production of body increase, based on the modification of the heat increment and metabolizable energy values as determined for maintenance, to conform to the ratios of the corresponding values (heat increment and metabolizable energy) of the mixed ration, for maintenance, to the same for body increase; (6) the use of a new procedure in determining the excess of energy expenditure of standing as compared with lying, in the computation of the heat production to conform to a standard day as to standing and lying, this factor being based on a recent and much improved consideration of this matter (*12*); and (7) to meet a special situation the average of the directly observed and the computed heat production (balance method) was used, in all instances in which both were available, in studying the energy metabolism as related to the plane of nutrition.

These several procedures are discussed in detail later in this paper, each in connection with the computations in which it is involved.

EXPERIMENTAL SUBJECTS

The steers used, which are designated Nos. 36 and 47, were unusually good subjects. They were of the same breed—Aberdeen-Angus—and had been thoroughly accustomed to experiments in the calorimeter through previous experience.

Steer No. 36 was calved on October 10, and steer No. 47 on August 29, both in the year 1924. During these experiments steer No. 36 was between 25 and 31 months, and steer No. 47 between 27 and 33 months, of age.

The steers were in good flesh, somewhat more than half fat, and came into the experimental program from a summer at pasture without grain feed.

They were of almost identical live weight. The average daily weight of each varied appreciably, from period to period, as the different planes of nutrition affected the "fill" and the true body weight, but the two steers agreed with each other in average daily weights in the several periods much more closely than ordinarily do consecutive daily weights of an individual steer.

EXPERIMENTAL DATA

The main foundation data of the experiments, as well as the corrections and computations necessary for their comparison and interpretation, are to be found in Tables 2 to 19, inclusive, while the derived final results are to be found in Tables 20 to 23. These tables will be discussed in numerical order.

TABLE 2.—*Digestibility of rations*

Period No.	Animal No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	N-free extract	Carbon	Energy	Nitrogen
			Gms.	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Cals.*	*Gms.*
1	47	Salt	30								
		Alfalfa hay	3,721	3,372.1	587.5	1,252.0	56.1	1,476.5	1,698.4	16,665.2	94.0
		Corn meal	3,663	3,605.7	411.2	80.4	148.1	2,966.0	1,689.0	16,701.9	65.8
		Total fed	7,414	6,977.8	998.7	1,332.4	204.2	4,442.5	3,387.4	33,367.1	159.8
		Feces	2,016	1,842.7	363.1	743.5	79.8	656.3	1,001.3	10,142.1	58.1
		Digestibility (per cent)	72.8	73.6	63.6	44.2	60.9	85.2	70.4	69.6	63.6
2	36	Salt	30								
		Alfalfa hay	3,545	3,212.6	559.7	1,192.8	53.4	1,406.7	1,618.1	15,877.0	89.5
		Corn meal	3,492	3,437.4	392.0	76.6	141.2	2,827.6	1,610.2	15,922.2	62.7
		Total fed	7,067	6,650.0	951.7	1,269.4	194.6	4,234.3	3,228.3	31,799.2	152.2
		Feces	1,973	1,797.1	360.2	720.3	76.8	639.8	965.0	9,794.3	57.6
		Digestibility (per cent)	72.1	73.0	62.2	43.3	60.5	84.9	70.1	69.2	62.2
3	47	Salt	30								
		Alfalfa hay	2,832	2,566.4	447.1	952.9	42.7	1,123.7	1,292.6	12,683.7	71.5
		Corn meal	2,785	2,741.4	312.6	61.1	112.6	2,255.1	1,284.2	12,698.5	50.0
		Total fed	5,647	5,307.8	759.7	1,014.0	155.3	3,378.8	2,576.8	25,382.2	121.5
		Feces	1,419	1,292.8	254.0	549.4	54.6	434.8	688.5	6,940.3	40.6
		Digestibility (per cent)	74.9	75.6	66.6	45.8	64.8	87.1	73.3	72.7	66.6
4	36	Salt	30								
		Alfalfa hay	2,698	2,445.0	426.0	907.8	40.7	1,070.5	1,231.5	12,083.5	68.2
		Corn meal	2,655	2,613.5	298.0	58.3	107.4	2,149.8	1,224.2	12,105.8	47.7
		Total fed	5,383	5,058.5	724.0	966.1	148.1	3,220.3	2,455.7	24,189.3	115.9
		Feces	1,371	1,232.6	247.0	531.9	58.0	395.7	659.9	6,668.8	39.5
		Digestibility (per cent)	74.5	75.6	65.9	44.9	60.8	87.7	73.1	72.4	65.9
5	47	Salt	30								
		Alfalfa hay	943	854.6	148.9	317.3	14.2	374.2	430.4	4,223.4	23.8
		Corn meal	920	905.7	95.3	20.5	35.8	754.1	426.1	4,193.8	15.2
		Total fed	1,893	1,760.3	244.2	337.8	50.0	1,128.3	856.5	8,417.2	39.0
		Feces	456	410.8	73.0	200.7	18.6	118.5	222.2	2,262.3	11.7
		Digestibility (per cent)	75.9	76.7	70.1	40.6	62.8	89.5	74.1	73.1	70.1
6	36	Salt	30								
		Alfalfa hay	949	860.0	149.8	319.3	14.3	376.6	433.2	4,250.3	24.0
		Corn meal	936	921.4	96.9	20.8	36.4	767.3	433.5	4,266.7	15.5
		Total fed	1,915	1,781.4	246.7	340.1	50.7	1,143.9	866.7	8,517.0	39.5
		Feces	467	424.3	71.1	220.3	17.9	115.0	229.1	2,326.5	11.4
		Digestibility (per cent)	75.6	76.2	71.2	35.2	64.7	89.9	73.6	72.7	71.2

TABLE 2.—*Digestibility of rations*—Continued

Period No.	Animal No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	N-free extract	Carbon	Energy	Nitrogen
7	47	Salt	30								
		Alfalfa hay	1,911	1,731.8	301.7	643.0	28.8	758.3	872.3	8,558.8	48.3
		Corn meal	1,879	1,849.7	194.6	41.8	73.1	1,540.2	870.3	8,565.3	31.1
		Total fed	3,820	3,581.5	496.3	684.8	101.9	2,298.5	1,742.6	17,124.1	79.4
		Feces	897	812.0	148.2	378.3	38.1	247.4	438.7	4,399.0	23.7
		Digestibility (per cent)	76.5	77.3	70.1	44.8	62.6	89.2	74.8	74.3	70.1
8	36	Salt	30								
		Alfalfa hay	1,902	1,723.6	300.3	640.0	28.7	754.6	868.1	8,518.5	48.0
		Corn meal	1,860	1,831.0	192.6	41.3	72.4	1,524.7	861.5	8,478.7	30.8
		Total fed	3,792	3,554.6	492.9	681.3	101.1	2,279.3	1,729.6	16,997.2	78.8
		Feces	900	806.5	144.1	380.8	36.1	245.5	441.5	4,466.0	23.0
		Digestibility (per cent)	76.3	77.3	70.8	44.1	64.3	89.2	74.5	73.7	70.8
9	47	Salt	30								
		Alfalfa hay	5,771	5,229.9	911.1	1,941.8	87.0	2,290.0	2,634.1	25,846.6	145.8
		Total fed	5,801	5,229.9	911.1	1,941.8	87.0	2,290.0	2,634.1	25,846.6	145.8
		Feces	2,339	2,117.5	297.5	1,111.2	84.6	624.2	1,130.2	11,244.8	47.6
		Digestibility (per cent)	59.7	59.5	67.3	42.8	2.8	72.7	57.1	56.5	67.3
10	36	Salt	30								
		Alfalfa hay	5,763	5,222.6	909.9	1,939.1	86.8	2,286.8	2,630.5	25,810.7	145.6
		Total fed	5,793	5,222.6	909.9	1,939.1	86.8	2,286.8	2,630.5	25,810.7	145.6
		Feces	2,334	2,109.1	30.1	1,091.6	84.7	631.7	1,130.1	11,280.9	48.2
		Digestibility (per cent)	59.7	59.6	66.9	43.7	2.4	72.4	57.0	56.3	66.9

The digestibility of the rations, as set forth in Table 2, is the usual apparent digestibility, representing the difference between the amounts of constituents in feed and feces.

The considerable length of the digestion periods and the high degree of regularity of the treatment given the animals were reflected in an unusually satisfactory regularity in the elimination of excreta; and the fact that the composition of the ration was the same in all but two periods, Nos. 9 and 10, constituted a favorable basis for criticism of results obtained. The digestion coefficients, therefore, are good figures of their sort.

The more noteworthy coefficients are those representing the rations of alfalfa hay alone, fed in periods 9 and 10, the digestibility of the nitrogen-free extract being decidedly low (72.4 to 72.7 per cent for the alfalfa hay, as compared with 84.7 to 89.9 per cent for the mixed ration) and that of the ether extract being almost negligible (2.4 to 2.8 per cent for the alfalfa hay, as compared with 60.5 to 64.8 per cent for the mixed ration).

These data call attention to the facts that ether extract includes a very great diversity of chemical compounds and that the apparent digestibility of ether extract may be a highly deceptive observation—especially under conditions such that the ether extract of the feces is largely of metabolic origin—thus not a feed residue.

The digestibility of the rations in relation to the plane of nutrition will be discussed in connection with the partition of the gross energy.

The quantities of carbon dioxide, water vapor, and methane given off by the experimental subjects during each calorimeter day are recorded in Table 3 as an exhibit of the degree of regularity, and therefore of reliability, characterizing these data which enter in important ways into the computation of the heat production and the metabolizable energy.

TABLE 3.—*Carbon dioxide, water vapor, and methane eliminated per day*

Animal No.	Period No.	Calorimeter day	Elimination of—				
			CO_2	C as CO_2	H_2O	CH_4	C as CH_4
			Grams	*Grams*	*Grams*	*Grams*	*Grams*
Steer 47	1	First	5,843.55	1,593.54	5,496.94	177.47	132.81
		Second	5,976.87	1,629.89	6,057.24	186.26	139.39
		Third	6,029.48	1,644.24	7,159.39	185.88	139.10
		Average	5,949.97	1,622.56	6,237.85	183.20	137.10
Steer 36	2	First	5,812.48	1,585.06	5,864.28	160.89	120.41
		Second	6,022.82	1,642.42	7,313.00	164.60	123.18
		Third	6,033.10	1,645.23	6,796.25	162.14	121.34
		Average	5,956.13	1,624.24	6,657.84	162.54	121.64
Steer 47	3	First	5,053.36	1,378.05	6,329.57	140.08	104.83
		Second	5,075.37	1,384.05	6,566.50	142.17	106.39
		Third	5,122.79	1,396.98	6,643.97	140.42	105.08
		Average	5,083.84	1,386.36	6,513.35	140.89	105.43
Steer 36	4	First	5,094.79	1,389.35	6,468.77	141.30	105.74
		Second	4,975.13	1,356.72	6,508.00	134.73	100.83
		Third	5,044.07	1,375.52	6,678.48	137.97	103.25
		Average	5,038.00	1,373.86	6,551.75	138.00	103.27
Steer 47	5	First	2,901.87	791.34	3,478.84	65.20	48.79
		Second	2,852.54	777.89	3,356.94	61.21	45.81
		Third	2,919.17	796.06	3,490.29	59.48	44.51
		Average	2,891.19	788.43	3,442.02	61.96	46.37
Steer 36	6	First	3,034.36	827.47	3,542.92	66.91	50.08
		Second	3,027.13	825.50	3,231.74	62.58	46.83
		Third	3,015.94	822.45	3,230.14	57.43	42.98
		Average	3,025.81	825.14	3,334.93	62.31	46.63
Steer 47	7	First	3,932.39	1,072.36	4,389.37	111.85	83.70
		Second	3,859.75	1,052.55	4,273.07	107.77	80.65
		Third	3,922.04	1,069.54	4,415.26	106.12	79.42
		Average	3,904.73	1,064.82	4,359.23	108.58	81.26
Steer 36	8	First	4,050.14	1,104.47	4,750.43	110.98	83.05
		Second	4,040.16	1,101.75	4,434.02	111.23	83.24
		Third	4,010.92	1,093.78	4,280.65	110.90	82.99
		Average	4,033.74	1,100.00	4,488.37	111.04	83.09
Steer 47	9	First	4,498.91	1,226.85	6,568.68	127.35	95.31
		Second	4,480.55	1,221.85	6,145.39	124.12	92.88
		Average	4,489.73	1,224.35	6,357.04	125.74	94.10
Steer 36	10	First	4,762.04	1,298.61	5,860.43	122.63	91.77
		Second	4,695.51	1,280.47	5,800.42	124 34	93.05
		Third	4,762.95	1,298.86	5,875.55	125.40	93.85
		Average	4,740.17	1,292.65	5,845.47	124.12	92.89

Calorimeter period No. 9 was terminated during the third day on account of refusal of feed; therefore the data are complete for only two days during this period. In all other cases the data cover three full days.

The agreement between the data for the individual days of each period was remarkably good, and in no complete day were the data considered unfit for inclusion in the computation of averages.

The water vapor was not measured in periods 1 and 2 on account of the fact that the heat production was not determined by the direct method during these periods; the data for water vapor, therefore, not being needed, as usual, for the determination of the latent heat of water vapor, and for the water balance.

Preliminary to the computation of the balance of matter an important correction, based on the nitrogen balance, was applied to the potential energy of the urine and of the body balance of protein.

In order to obtain a correct value of the metabolizable energy of the ration it is necessary to consider the gross energy of the body gain as metabolizable, except for that portion of the energy of the protein gained which would appear unoxidized in the urine if the protein gained had all been katabolized. From the point of view of energy metabolism, therefore, a part of the energy of protein stored is not metabolizable.

Likewise, in case of a loss of protein from the body, the metabolizable energy of this protein is less than its gross energy by that quantity appearing unoxidized in the urine.

The metabolizable energy of protein either gained or lost, therefore, is less than its gross energy by the amount of the energy of the urinary constituents which would result from the katabolism of this protein.

The amount of such minus corrections of the metabolizable energy, on account of the nonmetabolizable fraction of the protein gained or lost, is used as a plus correction of the energy of the urine in case of body gain of protein, and as a minus correction of the same in case of body loss of protein.

The gross or potential energy of body protein is obtained by multiplying its amount in grams by the factor 5.7; and the correction for the nonmetabolizable fraction of such protein is computed by multiplying the body gain or loss of nitrogen, in grams, by the factor 7.45.

TABLE 4.—*Energy of the urine and of the protein corrected for the incomplete oxidation of protein gained or lost*

Period No.	Balance of N	Correction (N×7.45)	Energy of urine: Uncorrected for N equilibrium	Energy of urine: Corrected	Energy of protein: Uncorrected (grams protein×5.7)	Energy of protein: Corrected
	Grams	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
1	+14.6	108.8	1,146.4	1,255.2	499.3	390.5
2	+11.2	83.4	1,133.6	1,217.0	383.0	299.6
3	+6.7	49.9	996.8	1,046.7	229.1	179.2
4	+5.5	41.0	1,001.7	1,042.7	188.1	147.1
5	−14.0	104.3	536.7	432.4	478.8	374.5
6	−13.8	102.8	566.0	463.2	472.0	369.2
7	+7.8	58.1	710.2	768.3	266.8	208.7
8	+8.7	64.8	737.2	802.0	297.5	232.7
9	+8.7	64.8	1,243.3	1,308.1	297.5	232.7
10	+8.4	62.6	1,260.9	1,323.5	287.3	224.7

The corrected values as given in Table 4 were employed in the computation of the balance of energy, as in Table 5.

TABLE 5.—*Balance of matter and energy per day*

PERIOD 1, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Alfalfa hay	3,721.0		94.0	1,698.4	16,665.2
Corn meal	3,663.0		65.8	1,689.0	16,701.9
Total	7,384.0		159.8	3,387.4	33,367.1
Outgo:					
Feces	2,016.0		58.1	1,001.3	10,142.1
Urine			87.1	129.6	1,255.2
Methane	183.2			137.1	2,444.6
Carbon dioxide	5,950.0			1,622.5	
Metabolizable:					
Income minus urine, feces, and methane					19,525.2
Body balances:					
Fat	+589.3			+450.9	+5,598.4
Protein	+87.6		+14.6	+46.0	+390.5
Computed heat production					13,536.3
PERIOD 2, STEER NO. 36					
Income:					
Alfalfa hay	3,545.0		89.5	1,618.1	15,877.0
Corn meal	3,492.0		62.7	1,610.2	15,922.2
Total	7,037.0		152.2	3,228.3	31,799.2
Outgo:					
Feces	1,973.0		57.6	965.0	9,794.3
Urine			83.4	125.3	1,217.0
Methane	162.5			121.6	2,168.4
Carbon dioxide	5,956.1			1,624.2	
Metabolizable:					
Income minus urine, feces, and methane					18,619.5
Body balances:					
Fat	+466.5			+356.9	+4,431.8
Protein	+67.2		+11.2	+35.3	+299.6
Computed heat production					13,888.1
PERIOD 3, STEER No. 47					
Income:					
Alfalfa hay	2,832.0	410	71.5	1,292.6	12,683.7
Corn meal	2,785.0	472	50.0	1,284.2	12,698.5
Water		15,713			
Total	5,617.0	16,595	121.5	2,576.8	25,382.2
Outgo:					
Feces	1,419.0	4,389	40.6	688.5	6,940.3
Urine		7,935	74.2	110.6	1,046.7
Methane	140.9			105.4	1,880.2
Carbon dioxide	5,083.9			1,386.4	
Water vapor		6,514			
Metabolizable:					
Income minus urine, feces, and methane					15,515.0
Body balances:					
Fat	+346.1			+264.8	+3,288.0
Protein	+40.2		+6.7	+21.1	+179.2
Water		−2,243			
Computed heat production					12,047.8
Observed heat production					11,692.9

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 4, STEER No. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Alfalfa hay	2,698.0	385	68.2	1,231.5	12,083.5
Corn meal	2,655.0	438	47.7	1,224.2	12,105.8
Water		12,723			
Total	5,353.0	13,546	115.9	2,455.7	24,189.3
Outgo:					
Feces	1,371.0	5,689	39.5	659.9	6,668.8
Urine		5,487	70.9	109.8	1,042.7
Methane	138.0			103.3	1,841.5
Carbon dioxide	5,038.0			1,373.9	
Water vapor		6,552			
Metabolizable:					
Income minus urine, feces, and methane					14,636.3
Body balances:					
Fat	+250.3			+191.5	+2,377.9
Protein	+33.0		+5.5	+17.3	+147.1
Water		−4,182			
Computed heat production					12,111.3
Observed heat production					11,854.1
PERIOD 5, STEER NO. 47					
Income:					
Alfalfa hay	943.0	121	23.8	430.4	4,223.4
Corn meal	920.0	173	15.2	426.1	4,193.8
Water		6,237			
Total	1,863.0	6,531	39.0	856.5	8,417.2
Outgo:					
Feces	456.0	853	11.7	222.2	2,262.3
Urine		4,351	41.3	55.1	432.4
Methane	62.0			46.4	827.3
Carbon dioxide	2,891.2			788.4	
Water vapor		3,442			
Metabolizable:					
Income minus urine, feces, and methane					4,895.2
Body balances:					
Fat	−276.4			−211.5	−2,625.8
Protein	−84.0		−14.0	−44.1	−374.5
Water		−2,115			
Computed heat production					7,895.5
Observed heat production					7,754.5
PERIOD 6, STEER NO. 36					
Income:					
Alfalfa hay	949.0	134	24.0	433.2	4,250.3
Corn meal	936.0	157	15.5	433.5	4,266.7
Water		9,073			
Total	1,885.0	9,364	39.5	866.7	8,517.0
Outgo:					
Feces	467.0	1,044	11.4	229.1	2,326.5
Urine		4,926	41.9	57.6	463.2
Methane	62.3			46.6	831.3
Carbon dioxide	3,025.8			825.2	
Water vapor		3,335			
Metabolizable:					
Income minus urine, feces, and methane					4,896.0
Body balances:					
Fat	−324.5			−248.3	−3,082.8
Protein	−82.8		−13.8	−43.5	−369.2
Water		+59			
Computed heat production					8,348.0
Observed heat production					8,155.8

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 7, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Alfalfa hay	1,911.0	225	48.3	872.3	8,558.8
Corn meal	1,879.0	313	31.1	870.3	8,565.3
Water		10,693			
Total	3,790.0	11,231	79.4	1,742.6	17,124.1
Outgo:					
Feces	897.0	2,795	23.7	438.7	4,399.0
Urine		5,650	47.9	77.7	768.3
Methane	108.6			81.3	1,449.2
Carbon dioxide	3,904.7			1,064.8	
Water vapor		4,359			
Metabolizable:					
Income minus urine, feces, and methane					10,507.6
Body balances:					
Fat	+72.5			+55.5	+688.8
Protein	+46.8		+7.8	+24.6	+208.7
Water		−1,573			
Computed heat production					9,610.1
Observed heat production					9,382.8

PERIOD 8, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	1,902.0	257	48.0	868.1	8,518.5
Corn meal	1,860.0	332	30.8	861.5	8,478.7
Water		9,119			
Total	3,762.0	9,708	78.8	1,729.6	16,997.2
Outgo:					
Feces	900.0	3,629	23.0	441.5	4,466.0
Urine		5,035	47.1	78.8	802.0
Methane	111.0			83.1	1,481.2
Carbon dioxide	4,033.7			1,100.0	
Water vapor		4,488			
Metabolizable:					
Income minus urine, feces, and methane					10,248.0
Body balances:					
Fat	−1.6			−1.2	−15.2
Protein	+52.2		+8.7	+27.4	+232.7
Water		−3.444			
Computed heat production					10,030.5
Observed heat production					9,839.7

PERIOD 9, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	5,771.0	677	145.8	2,634.1	25,846.6
Water		12,445			
Total	5,771.0	13,122	145.8	2,634.1	25,846.6
Outgo:					
Feces	2,339.0	6,777	47.6	1,130.2	11,244.8
Urine		8,484	89.5	141.0	1,308.1
Methane	125.8			94.1	1,678.7
Carbon dioxide	4,489.8			1,224.4	
Water vapor		6,357			
Metabolizable:					
Income minus urine, feces, and methane					11,615.0
Body balances:					
Fat	+22.2			+17.0	+210.9
Protein	+52.2		+8.7	+27.4	+232.7
Water		−8,496			
Computed heat production					11,171.4
Observed heat production					11,254.6

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 10, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Alfalfa hay	5,763.0	613	145.6	2,630.5	25,810.7
Water		17,410			
Total	5,763.0	18,023	145.6	2,630.5	25,810.7
Outgo:					
Feces	2,334.0	7,491	48.2	1,130.1	11,280.9
Urine		7,689	89.0	143.5	1,323.5
Methane	124.1			92.9	1,656.0
Carbon dixoide	4,740.2			1,292.7	
Water vapor		5,846			
Metabolizable:					
Income minus urine, feces, and methane					11,550.3
Body balances:					
Fat	−72.1			−55.2	−685.0
Protein	+50.4		+8.4	+26.5	+224.7
Water		−3,003			
Computed heat production					12,010.6
Observed heat production					11,635.0

It is helpful in grasping the significance of this correction to realize that it distinguishes, in any case, between the gross energy of protein and that portion of its energy which is available to the animal.

In Table 5 are given the average daily income and outgo of water, nitrogen, carbon, and energy; the metabolizable energy of the ration; and the body balances of fat, protein, energy, and water; also, for convenience in the use of these data, the amounts of both the computed and the observed heat production, in all but two periods, are given.

It will be noted that in all cases in which there are values for both the observed and the computed heat production, except in period 9, the computed value is slightly higher than the observed, as measured by direct calorimetry. The cause of this difference was not determined, and no satisfactory basis for choice between the two values was established. After carefully weighing the various possibilities of error in both methods of determination of the heat production, it was concluded that, pending the accomplishment of certain improvements in the mechanical accessories of the calorimetric equipment, the values of the computed and the directly observed heat production should be averaged.

Three important corrections of the heat production were made preparatory to its final use.

The first, shown in Table 6, is a correction of the observed heat production, based on the body gain or loss of protein, fat, and water, for the purpose of correcting for the gain or loss of sensible heat by the body of the animal while in the calorimeter. This correction is necessitated by the fact that in case of gain of these components heat is stored which has not been measured, and, in case of loss, heat is liberated as these components cool to the chamber temperature, the measured heat being in excess by the quantity so lost.

In Table 6 is also given the heat emission, by radiation and conduction, as observed, for subperiods of 12 hours each, there being six such intervals in each experimental period. A comparison of these

TABLE 6.—*Heat emission and heat production per day*

Animal No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Correction for body gain	Heat production
				By radiation and conduction	As latent heat of water vapor	Total		
				Calories	*Calories*	*Calories*	*Calories*	*Calories*
47	3		1	3,840.89				
			2	4,324.13				
		First		8,165.02	3,505.53	11,670.55		
			3	3,776.85				
			4	4,283.24				
		Second		8,060.09	3,645.32	11,705.41		
			5	4,002.95				
			6	4,154.35				
		Third		8,157.30	3,691.02	11,848.32		
		Daily average		8,127.47	3,613.96	11,741.43	−48.6	11,692.9
36	4		1	4,181.07				
			2	4,204.62				
		First		8,385.69	3,763.47	12,149.16		
			3	3,967.49				
			4	4,161.41				
		Second		8,128.90	3,719.36	11,848.26		
			5	3,963.57				
			6	4,186.72				
		Third		8,150.29	3,707.84	11,858.13		
		Daily average		8,221.63	3,730.22	11,951.85	−97.7	11,854.1
47	5		1	2,918.25				
			2	2,826.33				
		First		5,744.58	2,045.56	7,790.14		
			3	2,924.36				
			4	2,827.10				
		Second		5,751.46	1,973.88	7,725.34		
			5	2,851.06				
			6	2,997.63				
		Third		5,848.69	2,052.29	7,900.98		
		Daily average		5,781.58	2,023.91	7,805.49	−50.9	7,754.5
36	6		1	3,009.35				
			2	3,099.69				
		First		6,109.04	2,083.24	8,192.28		
			3	2,926.27				
			4	3,214.15				
		Second		6,140.42	1,900.26	8,040.68		
			5	3,070.11				
			6	3,276.77				
		Third		6,346.88	1,899.32	8,246.20		
		Daily average		6,198.78	1,960.94	8,159.72	−3.9	8,155.8
47	7		1	3,407.64				
			2	3,443.52				
		First		6,851.16	2,580.95	9,432.11		
			3	3,429.89				
			4	3,370.37				
		Second		6,800.26	2,512.57	9,312.83		
			5	3,437.63				
			6	3,468.72				
		Third		6,906.35	2,596.18	9,502.53		
		Daily average		6,852.59	2,563.23	9,415.82	−33.0	9,382.8
36	8		1	3,654.87				
			2	3,684.66				
		First		7,339.53	2,793.25	10,132.78		
			3	3,592.45				
			4	3,684.71				
		Second		7,277.16	2,607.20	9,884.36		
			5	3,618.55				
			6	3,590.41				
		Third		7,208.96	2,517.02	9,725.98		
		Daily average		7,275.22	2,630.15	9,914.37	−74.7	9,839.7

TABLE 6.—*Heat emission and heat production per day*—Continued

Animal No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Correction for body gain	Heat production
				By radiation and conduction	As latent heat of water vapor	Total		
				Calories	*Calories*	*Calories*	*Calories*	*Calories*
47	9		1	4,386.29				
			2	4,161.40				
		First		8,547.69	3,088.58	11,636.27		
			3	4,134.76				
			4	4,082.61				
		Second		8,217.37	3,026.96	11,244.33		
		Daily average		8,382.53	3,057.77	11,440.30	−185.7	11,254.6
36	10		1	4,086.53				
			2	4,365.98				
		First		8,452.51	3,445.99	11,898.50		
			3	3,907.65				
			4	4,149.88				
		Second		8,057.53	3,410.64	11,468.17		
			5	3,921.35				
			6	4,360.02				
		Third		8,281.37	3,454.82	11,736.19		
		Daily average		8,263.80	3,437.15	11,700 95	−66.0	11,635.0

subperiod data is not without interest, as revealing the character, as to uniformity, of the data entering into the determination of the average daily heat production. No close comparison of these subperiod values can be made, however, especially because they represent different proportionate intervals of time spent in the standing and the lying positions. The latent heat of water vapor is given, not for subperiods, but for experimental days.

The second and third of the above-mentioned corrections were applied to the average of the final observed and computed values for the heat production. These were a correction (Table 7) based on the duration of time spent by the animal in the standing and the lying positions, and the difference in energy expenditure of standing and lying—for use in the computation of the heat production of the standard day, in which the animal stood and lay down 12 hours each; and a correction (Table 18) for difference in the maintenance requirement of net energy, in the several experimental periods, due to difference in live weight, this correction being devised to permit the comparison of the heat production of the animal on different planes of nutrition.

In computing the standing and lying correction the difference between the aggregate of intervals of time spent by the animal in the standing position, as compared with the 12-hour standard, is multiplied by a factor representing the amount by which the energy cost of standing, per unit of time, is greater than that of lying—this factor being adjusted to the live weight of the animal. The derivation of this factor is discussed in detail in a recent paper in this Journal (*12*) by Forbes, Kriss, Braman, and associates.

It should be noted that this correction, as determined for the steers used in this series of experiments, is materially greater than that used (*13*) for the same purpose in other recent papers from this institute. It seems that, for the present at least, determinations of this correction will have to be made in connection with each experimental program.

TABLE 7.—*Correction of heat production to a standard day of 12 hours standing and 12 hours lying*

Period No.	Steer No.	Time spent standing	Difference from 12 hours	Factor	Correction	Heat production		Heat observed or computed
						Uncorrected	Corrected	
		Hours	*Hours*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	
1	47	8.5	3.5	73.4	256.9	13,536.3	13,793.2	Computed.
2	36	13.4	1.4	72.9	−102.1	13,888.1	13,786.0	Do.
3	47	7.9	4.1	74.7	306.3	11,692.9	11,999.2	Observed.
						12,047.8	12,354.1	Computed.
4	36	10.4	1.6	74.0	118.4	11,854.1	11,972.5	Observed.
						12,111.3	12,229.7	Computed.
5	47	7.5	4.5	71.7	322.7	7,754.5	8,077.2	Observed.
						7,895.5	8,218.2	Computed.
6	36	8.9	3.1	71.2	220.7	8,155.8	8,376.5	Observed.
						8,348.0	8,568.7	Computed.
7	47	6.9	5.1	73.2	373.3	9,382.8	9,756.1	Observed.
						9,610.1	9,983.4	Computed.
8	36	9.5	2.5	72.7	181.8	9,839.7	10,021.5	Observed.
						10,030.5	10,212.3	Computed.
9	47	7.3	4.7	75.3	353.9	11,254.6	11,608.5	Observed.
						11,171.4	11,525.3	Computed.
10	36	12.8	.8	75.5	−60.4	11,635.0	11,574.6	Observed.
						12,010.6	11,950.2	Computed.

DETERMINATION OF THE FASTING KATABOLISM AS A MEASURE OF THE MAINTENANCE REQUIREMENT OF NET ENERGY

The directly determined fasting katabolism was adopted by this institute as the measure of the maintenance requirement of net energy of cattle in 1925, this departure from the earlier procedure (*1*) and the data upon which it rested being published by Forbes in a paper read before the American Society of Animal Production (*6*).

Further notice of the adoption of this measure—new as applied to cattle—was given by Forbes in Science (*5*); and a more extended study of the same matter, with additional experimental data, was published by Forbes, Kriss, and Braman in 1927 (*11*).

In the nature of the case, however, this measure can not be regarded as an absolute value; and its use in studies of the nutrition of cattle depends on the exact method by which it (the directly determined fasting katabolism) is determined.

Since the date of adoption of this measure, therefore, the conventional standardization of procedure in its determination has been a continuing problem at this institute, and the present discussion may be considered—apart from its relation to the main study in hand—as a further contribution to the subject of standardization of this determination.

The principal problem in regard to this measurement is as to the exact conditions under which the heat production of the animal is to be considered representative of true fast—a problem resulting, in the main, from the facts as to the complicated anatomy and physiology of the ruminant alimentary tract—a situation which was discussed at some length in the last paper (*11*) above referred to.

As a background condition it is the practice at this institute to determine the fasting katabolism only after a period of feeding on a plane of energy equilibrium, of sufficient length presumably to have accomplished the adjustment of the animal to this regimen. Under these conditions, if there is a carrying over of any habit or influence as to energy metabolism from the previous state of nutrition into the days of fast during which the fasting katabolism is measured, then this carrying over will be, in all cases, at least of similar origin and degree.

In the present experiments the sequence of treatments was such that both steers were on a plane of approximate energy equilibrium for 40 days immediately before the fasting periods; and during the last day of feeding only grain was given, roughage being withheld, with the idea that this procedure might assist in freeing the paunch from feed.

The steers were then further prepared for the measurement of the fasting katabolism by the administration of a physic. The preparation used was composed of sodium sulphate 12 parts, capsicum 2 parts, ginger 2 parts, and gamboge 1 part; and the treatment was two ½-pound doses, separated by a two-hour interval, on two successive mornings.

TABLE 8.—*Carbon dioxide, oxygen, and nitrogen in ingoing air during fast*

Animal, period, and subperiod Nos.	Aliquot, ingoing air	Carbon dioxide in aliquot	Carbon dioxide in aliquot	Carbon dioxide	Oxygen	Nitrogen
	Liters	*Grams*	*Liters*	*Per cent*	*Per cent*	*Per cent*
Steer No. 47, period 11:						
Subperiod 1	446.75	0.2899	0.1467	0.033	[a] 20.935	79.032
Subperiod 2						
Subperiod 3	449.44	.2704	.1368	.030	20.935	79.035
Subperiod 4	447.36	.2800	.1417	.032	20.935	79.033
Subperiod 5				.031	20.935	79.034
Subperiod 6	444.66	.2648	.1340	.030	20.935	79.035
Subperiod 7	449.15	.2621	.1326	.030	20.935	79.035
Subperiod 8	447.44	.2629	.1330	.030	20.935	70.035
Steer No. 36, period 12:						
Subperiod 1	435.22	.2750	.1391	.032	20.929	79.039
Subperiod 2	431.73	.2785	.1409	.033	20.937	70.030
Subperiod 3	433.69	.2837	.1435	.033	20.932	79.035
Subperiod 4	433.33	.2782	.1407	.032	20.936	79.032
Subperiod 5	425.82	.2752	.1392	.033	20.940	79.027
Subperiod 6	437.77	.2847	.1440	.033	20.935	79.032
Subperiod 7	438.30	.2709	.1370	.031	20.936	79.033
Subperiod 8	435.42	.2748	.1390	.032	20.934	79.034

[a] Average of determinations for period 12.

TABLE 9.—*Carbon dioxide and oxygen in outcoming air during fast*

Animal, period, and subperiod Nos.	Outcoming air	Carbon dioxide	Carbon dioxide	Carbon dioxide	Oxygen
	Liters	*Grams*	*Liters*	*Per cent*	*Per cent*
Steer No. 47, period 11:					
Subperiod 1	323,282.8	1,463.8	740.5	0.229	20.678
Subperiod 2					
Subperiod 3	324,112.5	1,316.4	666.0	.205	20.698
Subperiod 4	324,399.2	1,380.0	698.1	.215	20.696
Subperiod 5	322,125.9	1,311.6	663.5	.206	20.719
Subperiod 6	322,342.1	1,332.1	673.9	.209	20.704
Subperiod 7	326,186.6	1,319.9	667.7	.205	20.706
Subperiod 8	326,640.2	1,309.6	662.5	.203	20.709
Steer No. 36, period 12:					
Subperiod 1	321,541.7	1,503.6	760.7	.237	20.685
Subperiod 2	321,235.3	1,479.1	748.3	.233	20.690
Subperiod 3	321,221.6	1,415.0	715.8	.223	20.692
Subperiod 4	322,791.1	1,392.6	704.5	.218	20.693
Subperiod 5	324,127.9	1,347.6	681.8	.210	20.700
Subperiod 6	324,391.1	1,370.1	693.1	.214	20.702
Subperiod 7	323,775.2	1,419.6	718.2	.222	20.692
Subperiod 8	324,485.1	1,341.4	678.6	.209	20.700

TABLE 10.—*Urinary nitrogen per 12-hour subperiod, and carbon dioxide, oxygen, and energy equivalents, during fast*

Animal and period Nos.	Average urinary nitrogen per 12-hour subperiod	Carbon dioxide equivalent (N×4.75)	Oxygen equivalent (N×5.94)	Energy equivalent (N×26.51)
	Grams	*Liters*	*Liters*	*Cals.*
Steer 47, period 11	18.7	88.8	111.1	495.7
Steer 36, period 12	21.1	100.2	125.3	559.4

TABLE 11.—*Heat production computed from the respiratory quotient, compared with the observed heat production (not corrected to the standard day), during fast*

Animal, period, and subperiod Nos.	Non-protein R. Q.	Corrected value per liter O_2	Total non-protein oxygen	Computed heat production			Observed heat production	Computed observed heat production
				Non-protein	Protein	Total		
Steer No. 47, period 11:		*Cals.*	*Liters*	*Cals.*	*Cals.*	*Cals.*	*Cals*	*Per cent*
Subperiod 1	0.706	4.680	772.0	3,617.6	495.7	4,113.3	4,533.0	90.7
Subperiod 2								
Subperiod 3	.673	4.686	710.3	3,328.5	495.7	3,824.2	3,703.5	103.3
Subperiod 4	.709	4.688	712.3	3,339.3	495.7	3,835.0	3,817.4	100.5
Subperiod 5	.766	4.759	619.6	2,948.7	495.7	3,444.4	3,630.2	94.9
Subperiod 6	.720	4.702	677.9	3,187.5	495.7	3,683.2	3,734.6	98.6
Subperiod 7	.706	4.686	682.5	3,198.2	495.7	3,693.9	3,616.7	102.1
Subperiod 8	.708	4.687	673.0	3,154.4	495.7	3,650.1	3,684.9	99.1
Steer No. 36, period 12:								
Subperiod 1	.807	4.809	692.5	3,330.2	559.4	3,889.6	4,315.6	90.1
Subperiod 2	.780	4.776	695.1	3,319.8	559.4	3,879.2	4,076.6	95.2
Subperiod 3	.741	4.728	688.1	3,253.3	559.4	3,812.7	3,993.1	95.5
Subperiod 4	.707	4.686	707.8	3,316.8	559.4	3,876.2	3,881.4	99.9
Subperiod 5	.670	4.686	706.7	3,311.6	559.4	3,871.0	3,731.7	103.7
Subperiod 6	.721	4.703	675.3	3,175.9	559.4	3,735.3	3,781.7	98.8
Subperiod 7	.732	4.717	707.9	3,339.2	559.4	3,898.6	4,071.3	95.8
Subperiod 8	.694	4.686	683.0	3,200.5	559.4	3,759.9	3,672.1	102.4

TABLE 12.—*Carbon dioxide, water vapor, and methane eliminated per day, during fast*

Animal and period No.	Subperiods, and days of fast	Carbon dioxide eliminated	Water vapor eliminated	Methane eliminated
		Grams	*Grams*	*Grams*
Steer 47, period 11	Subperiod 1	1,259.36	2,681.72	0.68
	Subperiod 2			
	Third day			
	Subperiod 3	1,108.55	1,898.26	.69
	Subperiod 4	1,180.17	2,043.88	
	Fourth day	2,288.72	3,942.14	
	Subperiod 5	1,115.18	1,818.62	.15
	Subperiod 6	1,146.36	1,667.65	.73
	Fifth day	2,261.54	3,486.27	.88
	Subperiod 7	1,120.16	1,529.19	.61
	Subperiod 8	1,125.89	1,471.76	.17
	Sixth day	2,246.05	3,000.95	.78
Steer 36, period 12	Subperiod 1	1,306.31	1,756.92	.37
	Subperiod 2	[a] 1,262.18	[a] 1,940.50	1.60
	Fourth day	2,568.49	3,697.42	1.97
	Subperiod 3	1,204.62	1,695.59	1.29
	Subperiod 4	1,192.51	1,550.64	.07
	Fifth day	2,397.13	3,246.23	1.36
	Subperiod 5	1,128.07	1,437.87	1.09
	Subperiod 6	1,159.46	1,376.25	.90
	Sixth day	2,287.53	2,814.12	1.99
	Subperiod 7	[a] 1,232.61	[a] 1,457.94	.79
	Subperiod 8	1,129.88	1,256.59	.82
	Seventh day	2,362.49	2,714.53	1.61

[a] Corrected for man entering chamber

TABLE 13.—*Heat emission and heat production per day, during fast*

Animal and period No.	Subperiod No.	Calorimeter temperature	Water consumption	Heat emission: By radiation and conduction	Heat emission: As latent heat of water vapor	Heat emission: Total	Correction for body gain	Heat production
		°C.	*Kgm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Steer 47, period 11	1	17.6	24.58	2,581.45	1,574.17	4,155.62	+377.38	4,533.00
	2							
	3	17.3	1.19	2,635.42	1,116.18	3,751.60	−48.06	3,703.54
	4	17.4		2,725.83	1,201.80	3,927.83	−110.21	3,817.42
	5	17.4	1.17	2,614.78	1,069.35	3,684.13	−53.97	3,630.16
	6	17.3		2,824.01	980.58	3,804.59	−69.97	3,734.62
	7	17.4	1.02	2,755.28	899.16	3,654.44	−37.75	3,616.69
	8	17.4		2,877.74	865.39	3,743.13	−58.24	3,684.89
Steer 36, period 12	1	17.4	8.22	3,182.83	1,033.07	4,215.90	+99.71	4,315.61
	2	17.4		2,992.12	1,141.01	4,133.13	−56.50	4,076.63
	3	17.2	3.04	3,074.28	997.01	4,071.29	−78.20	3,993.09
	4	17.1		3,107.02	911.78	4,018.80	−137.43	3,881.37
	5	17.1	1.79	2,924.43	845.47	3,769.90	−38.18	3,731.72
	6	17.2		3,057.85	809.24	3,867.09	−85.44	3,781.65
	7	17.3	4.94	3,212.71	857.27	4,069.98	+1.34	4,071.32
	8	17.3		3,007.96	738.87	3,746.83	−74.76	3,672.07

TABLE 14.—*Heat production of fasting steers, corrected to the standard day*

Animal and period No. and days of fast	Weight of animal	Time spent standing	Difference from standard of time spent standing	Correction [a]	Heat production, uncorrected	Heat production, corrected to standard day
Steer 47, period 11:	*Kgm.*	*Hours*	*Hours*	*Cals.*	*Cals.*	*Cals.*
Fourth day	478.6	6.77	5.23	+378.13	7,520.96	7,899.09
Fifth day	478.6	6.10	5.90	+426.57	7,364.78	7,791.35
Sixth day	478.6	6.77	5.23	+378.13	7,301.58	7,679.71
Average						7,790.01
Steer 36, period 12:						
Fourth day	467.8	10.57	1.43	+101.10	8,392.24	8,493.34
Fifth day	467.8	6.65	5.35	+378.25	7,874.46	8,252.71
Sixth day	467.8	4.90	7.10	+501.97	7,513.37	8,015.34
Seventh day	467.8	9.60	2.40	+169.68	7,743.39	7,913.07
Average of last three days						8,060.37

[a] Correction for steer No. 47, 72.3 Cals. per hour; for steer No. 36, 70.7 Cals. per hour.

TABLE 15.—*Contents of alimentary tract of steers after fast*

Animal No.	Contents of—: Paunch and reticulum	Omasum	Abomasum	Small intestine	Large intestine	Total, alimentary tract
	Kilograms dry matter					
Steer 47, after 6 days' fast	0.894	0.175	0.229	0.191	0.248	1.737
Steer 36, after 7 days' fast	.517	.281	.089	.151	.089	1.127
	Kilograms fresh substance					
Steer 47, after 6 days' fast	12.100	1.045	2.041	2.392	2.184	19.762
Steer 36, after 7 days' fast	11.285	1.665	1.227	3.015	1.008	18.200

The results of the study of the fasting katabolism are given in Tables 8 to 15, this study, including analysis of the air as it entered and as it left the calorimeter chamber (Tables 8 and 9), determinations of the urinary nitrogen (Table 10), comparisons of the heat production computed from the respiratory quotient with the directly observed heat production (Table 11), data for the carbon dioxide, water vapor, and methane eliminated per day (Table 12), for the heat emission and the heat production per day (Table 13), and for the heat production computed to the standard day (Table 14); also (Table 15) weights of the contents of the alimentary tract after fast.

The measurement of the fasting katabolism (Table 13) began on the third day of fast with steer No. 47, and on the fourth day with steer No. 36.

The third day of fast with steer No. 47, however (subperiods 1 and 2), proved to be too soon after the preparatory treatment for the satisfactory handling of the animal in the calorimeter, and the data for this day were incomplete; and with steer No. 36 the heat production on the fourth day of fast (subperiods 1 and 2) was considerably higher than on the three following days; also the nonprotein respiratory quotients for the two subperiods of this day (0.807 and 0.780; see Table 11) show that a true state of fast had not been reached. The data used, as measures of the fasting katabolism, therefore, were, in the case of steer No. 47, from the fourth, fifth, and sixth days of fast, and with steer No. 36, from the fifth, sixth, and seventh days of fast.

The standardization of procedures in this matter of treatment preliminary to the measurement of the fasting katabolism has not yet been established in all details.

There has been a suggestion, in the course of the heat production on the successive days in the calorimeter during fast, that even after the usual 14-hour preliminary interval another day must elapse before the animal is completely adjusted and resigned to the experimental program; and it is quite conceivable that a longer period of adjustment is necessary with the fasting animal than with the animal which receives feed.

In addition to the course of the heat production during fast, we have, as bases for judgment as to when the animal is in a true postresorptive state, the conditions as to the gaseous metabolism, and the content of feed residues in the alimentary tract.

The conditions of especial interest with reference to the gaseous metabolism are as relating to the nonprotein respiratory quotient, and to the methane elimination, the former as an indication of the time of exhaustion of the supply of nutriment from the alimentary tract, and also of the status of the glycogen reserve—the time, therefore, at which the animal becomes dependent exclusively on body fat and protein as sources of energy; and the latter—the methane elimination—as an indication of the time at which the fermentation of feed residues in the alimentary tract, with resultant liberation of heat and assimilable nutriment, diminishes to become a negligible factor.

The conditions necessary for the determination of the respiratory quotient were not quite ideal, in that it was necessary to collect the air sample for oxygen estimation over water; but results of significance were obtained.

Carbon dioxide was determined gravimetrically by absorption from measured, continuous aliquots of both ingoing and outcoming air; and oxygen was determined, by means of the Sondén apparatus, in a large, continuous, 12-hour aspirator sample.

The determinations of oxygen in the ingoing air (Table 8) were made in samples of the outside air taken three times per 12-hour subperiod, and are nearly enough in agreement with the known content of oxygen in out-of-door air to show that satisfactory conditions of analysis prevailed.

Also the heat production as computed by the use of respiratory quotients, derived from the analytical data in Tables 8 and 9, shows by comparison with the directly observed heat production, as in Table 11, that the respiratory quotients are essentially correct.

An examination of these nonprotein respiratory quotients indicates that with steer No. 47 a state of true fast existed from the first day of measurement (the third day of fast); but with steer No. 36 the respiratory quotients indicated some oxidation of carbohydrate until the second subperiod (subperiod 4) of the fifth day of fast. It seems possible that there is an appreciable measure of individuality in cattle as to the duration of time necessary to the exhaustion of glycogen reserves.

The high respiratory quotients with steer No. 47 in subperiods 5 and 6, and with steer No. 36 in subperiods 6 and 7, in both cases after the respiratory quotient had been down to a level representative of true fast, may be the result of some imperfection of technic.

The number of grams of carbon dioxide and methane, of carbon in each of these, and of water vapor, given off by the steers in each subperiod during fast, are given in Table 12, these data being used in the computation of the heat emission and heat production, as set forth in Table 13.

The figures representing methane (Table 12) are of especial interest, since from the beginning of the observations during fast (on the third and the fourth days of fast, respectively, with the two steers) the methane produced weighed but a fraction of a gram per 12-hour subperiod—an exceedingly small quantity, in relation to the size of the animal and to the normal methane production during feeding (see Table 5: 162.5 to 183.2 gm. per 24 hours, during periods 1 and 2).

All considered, it seems quite feasible to get a steer into a state of essentially complete fast; but more evidence than is now at hand will be necessary to the final standardization of the method of doing so.

The heat emission by radiation and conduction, and as latent heat of water vapor, the correction for body loss (or gain), and final value for heat production comprise Table 13.

An interesting situation with respect to the method of heat outgo during the progress of fast is revealed by the data for heat emission by radiation and conduction as compared with the parallel figures for latent heat of water vapor. The former remains at about the same level, from subperiod to subperiod, while the latter decreases materially, in 10 among 13 consecutive pairs of subperiods.

The method of experimentation was not such as definitely to reveal the reason for this decrease in latent heat of water vapor, but possible decrease of respiration and of peripheral circulation and the observed decrease of water intake are suggested as causative factors.

Such a tendency toward a regular decrease in latent heat of water vapor as characterized these fasting periods is not apparent in some of the previous fasting experiments of this institute.

The prominent decrease of water intake during fast was probably due to decreased need for water, perhaps to an increased sensitiveness to heat loss, as through the drinking of water at a temperature lower than that of the body.

In the light of a recent study at this institute (*7*) it is the opinion of the writers that the steers were at no time subjected to subcritical temperatures.

About an hour and a half after the termination of the fasting calorimetric periods the steers were killed in order to permit the quantitative accounting for the feed residues in the alimentary tract, as indicating the validity of conditions for the fasting heat measurement, and to make possible the direct determination of the area of the hide as a measure of the surface area of the animal.

The dry matter of the feed residues found in the alimentary tract of steer No. 47 was 1.737 kgm., and of steer No. 36, 1.127 kgm. (Table 15). Of these quantities, however, only 29 and 38 per cent, respectively, were present in the true stomach (abomasum) and intestines. The remaining 71 and 62 per cent, respectively, were found in the paunch, reticulum, and omasum, from which there is little or no absorption of nutrients. The feed present in these portions of the alimentary tract, therefore, was negligible from the point of view of the present interest; that is, as available nutriment, or as contemporary feed residues.

These observations show that these steers were unable completely to empty the paunch, even in the course of a seven-day fast, but that conditions within the alimentary tract, at the termination of the fasting heat measurement, represented practically complete fast.

DETERMINATION OF THE SURFACE AREA OF THE EXPERIMENTAL SUBJECTS

The most significant conventional base value thus far proposed, to which to relate the maintenance requirement of cattle, is the surface area; but this value is not easily determined.

TABLE 16.—*Measurements of surface area of steers after fast*

Steer No.	Measured area of hide	Computed by Moulton's formula	Computed by Hogan's formula
	Sq. meters	*Sq. meters*	*Sq. meters*
47	(a) 4.931 (b) 4.882	5.301	5.009
36	4.950	5.305	4.763

In Table 16 are estimations of the surface area of the two steers, determined (1) by the measurement of the removed hide; (2) by Moulton's formula (*21*), $A = 0.1186\ W^{5/8}$, in which A is the surface area in square meters, and W the warm empty weight in kilograms; and by Hogan's formula (*15*), $S = W^{0.4} \times L^{0.6} \times K$, in which S is the surface area in square centimeters, W is the weight in kilograms, L is the length of the body in centimeters, and K is the constant, 217, for cattle.

The satisfactory use of Moulton's formula would require the further standardization of conditions and procedure in computing the empty weight. In the present use of this formula the empty weight was computed from the gross live weight during maintenance feeding, by multiplying by 0.9, as proposed by Moulton.

Direct measurements of the surface area of cattle have been made by Brody (*4*), by means of a so-called surface integrator; but it seemed impracticable thus to measure the surface area of the steers used in these experiments on account of their thick, wooly coats and their nervous disposition.

The most satisfactory method for determining the surface area of cattle seemed to the authors to be the measurement of the area of the removed hide. Accordingly, the freshly removed hide of each of the two steers used in these experiments was smoothed out, without stretching, on a concrete floor, and the outline drawn with chalk.

The main part of the area so inclosed was ruled into rectangles, and measured; and tracings were made, on paper, of the irregular areas at the edges.

The areas of these tracings were then measured with a planimeter; and, for comparison, their area was also computed from the weight of the pieces inclosed by the tracings in relation to that of a measured square of the same paper.

These two methods of measurement of the area of the tracings gave results agreeing satisfactorily; and the second was found much the easier to employ. The two values for the surface area of steer No. 47, designated (a) and (b), were both obtained by this second method of measurement.

This direct determination of the area of the removed hide, therefore, has been adopted as the most accurate and practicable measure of the surface area of cattle—for use in experiments in which the life of the subjects may be sacrificed.

The surface area of the steers, as thus determined by the measurement of the hides after fast, was made the basis of a computation of the surface area during the preceding maintenance period, by the following procedure:

First, the empty body weight during maintenance was computed by adding to the empty body weight after fast, as directly determined by slaughter test, the loss in body weight during fast, as computed from the loss in carbon and nitrogen.

Then the surface area of the steers at the maintenance level was computed by increasing the surface area (hide measurement) during fast in proportion as the five-eighths power of the computed body weight during maintenance was greater than the five-eighths power of the observed empty body weight after fast, this computation being based on Moulton's formula (*21*) for determining the surface area from the empty body weight.

The maintenance requirement of net energy was finally computed by multiplying the number of square meters of surface area during maintenance by the number of Calories of heat production per square meter of body surface as determined during fast. The data involved in these computations are given in Table 17.

TABLE 17.—*Derivation of values for surface area and maintenance requirement of net energy during the maintenance period preceding fast*

Animal No.	Surface area during fast	Fasting katabolism		Empty weight of fasting animals	Loss of body tissue after maintenance until end of fast	Computed empty weight in maintenance period	Computed surface area in maintenance period	Maintenance requirement of net energy per head
		Total	Per square meter of body surface					
	Sq. meters	*Cals.*	*Cals.*	*Kgm.*	*Kgm.*	*Kgm.*	*Sq. meters*	*Cals.*
47	4.906	7,790	1,588	444.8	13.8	458.6	5.000	7,940
36	4.950	8,060	1,628	436.5	16.8	453.3	5.068	8,251

FINAL COMPUTATIONS

The results of the final computations based on the experimental data, for the purpose of bringing out the facts as to the utilization of the energy of the feed, comprise Tables 18 to 23. The objects of these computations and the methods of making them will be briefly explained in the following paragraphs, and the results will be discussed later, under several appropriate headings.

TABLE 18.—*Heat production corrected to a basis of uniform live weight and maintenance requirement*

Animal No. and plane of nutrition	Period No.	Average live weight	Fasting katabolism (maintenance requirement)	Correction to be applied to heat production	Heat production			
					Uncorrected for live weight		Corrected to uniform live weight	
					Observed	Computed	Observed	Computed
		Kgm.	*Cals.*[a]	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Steer No. 47:								
Fast	11	478.6	7,790		7,790		7,790	
Half less than maintenance	5	474.8	7,749	+41	8,077	8,218	8,118	8,259
Maintenance (mixed ration)	7	484.8	7,857	−67	9,756	9,983	9,689	9,916
Half more than maintenance	3	494.6	7,963	−173	11,999	12,354	11,826	12,181
Twice maintenance	1	486.2	7,872	−82		13,793		13,711
Maintenance (hay only)	9		[b] 7,940	−150	11,609	11,525	11,459	11,375
Steer No. 36:								
Fast	12	467.8	8,060		8,060		8,060	
Half less than maintenance	6	471.2	8,099	−39	8,377	8,569	8,338	8,530
Maintenance (mixed ration)	8	481.2	8.213	−153	10,022	10,212	9,869	10,059
Half more than maintenance	4	490.2	8,315	−255	11,973	12,230	11,718	11,975
Twice maintenance	2	482.9	8,232	−172		13,786		13,614
Maintenance (hay only)	10		8,251	−191	11,575	11,950	11,384	11,759

[a] Computed to correspond to the average live weight of fasting in proportion to the two-thirds power of the live weights.

[b] Correction based on estimated loss of body tissue.

TABLE 19.—*Heat production, corrected for differences in live weight, and heat increments derived by comparison of the heat production in the periods of feeding with that of fast*

Animal and period No.	Heat production			Fasting katabolism	Heat increments	
	Observed	Computed	Average		Total	Per kilogram of dry matter
	Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Steer No. 47:						
Period 1		13,711	13,711	7,790	5,921	802
Period 3	11,826	12,181	12,004	7,790	4,214	750
Period 5	8,118	8,259	8,189	7,790	399	214
Period 7	9,689	9,916	9,803	7,790	2,013	531
Period 9	11,459	11,375	11,417	7,790	3,627	628
Steer No. 36:						
Period 2		13,614	13,614	8,060	5,554	789
Period 4	11,718	11,975	11,847	8,060	3,787	707
Period 6	8,338	8,530	8,434	8,060	374	198
Period 8	9,869	10,059	9,964	8,060	1,904	506
Period 10	11,384	11,759	11,572	8,060	3,512	609

TABLE 20.—*Partition of energy of feed*

Animal and period No.	Dry matter of feed mixture	Energy per kilogram of dry matter							
		Gross	Digestible	Metabolizable	Total net [a]	Feces	Heat increment	Methane	Urine
	Kgm.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*[b]	*Cals.*	*Cals.*
Steer 47:									
Period 1	7.384	4,519	3,145	2,644	1,842	1,374	802	331	170
Period 3	5.617	4,519	3,283	2,762	2,012	1,236	750	335	186
Period 7	3.790	4,518	3,358	2,772	2,241	1,161	531	382	203
Period 5	1.863	4,518	3,304	2,628	2,414	1,214	214	444	232
Steer 36:									
Period 2	7.037	4,519	3,127	2,646	1,857	1,392	789	308	173
Period 4	5.353	4,519	3,273	2,734	2,027	1,246	707	344	195
Period 8	3.762	4,518	3,331	2,724	2,218	1,187	506	394	213
Period 6	1 885	4,518	3,284	2,597	2,399	1,234	198	441	246

[a] Mixed values derived by the use of heat increments computed as in Table 19. Net-energy values for maintenance and for production are given in Tables 21 and 22.

[b] Based on the average of the observed and computed heat production corrected for differences in live weight, and computed as in Table 19.

TABLE 21.—*Net-energy values for maintenance*

Period No.	Steer No.	Dry matter of feed eaten			Total heat production	Fasting katabolism	Heat increments		Metabolizable energy per kilogram of dry matter	Net energy per kilogram of dry matter (for maintenance)	Utilization of metabolizable energy (for maintenance)
		Alfalfa hay	Corn meal	Total			Total	Per kilogram of dry matter			
		Kgm.	*Kgm.*	*Kgm.*	*Cals.*[a]	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
9	47	5.771		5.771	11,417	7,790	3,627	628	2,013	1,385	68.80
10	36	5.763		5.763	11,572	8,060	3,512	609	2,004	1,395	69.61
7	47	1.911	1.879	3.790	9,803	7,790	2,013	531	2,772	2,241	80.84
8	36	1.902	1.860	3.762	9,964	8,060	1,904	506	2,724	2,218	81.42
Computed values of corn meal	47							433	3,544	3,111	87.78
Do	36							401	3,460	3,059	88.41

[a] Average of the observed and computed heat production corrected for differences in live weight.

TABLE 22.—*Net-energy values of ration, or component, for body increase*

Steer No.	Periods compared	Ration or component	Planes of nutrition compared	Heat increment per kilogram of dry matter	Metabolizable energy per kilogram of dry matter in supermaintenance periods	Net energy per kilogram of dry matter (for body increase)	Utilization of metabolizable energy (for body increase)
		Kgm.		*Cals.*	*Cals.*	*Cals.*	*Per cent*
47	1 and 7	Hay and meal.	Twice maintenance and maintenance.	1,087	2,644	1,557	58.89
36	2 and 8	do	do	1,115	2,646	1,531	57.86
47	3 and 7	do	Half more than maintenance and maintenance.	1,205	2,762	1,557	56.37
36	4 and 8	do	do	1,184	2,734	1,550	56.69
47	1 and 7	Alfalfa	Twice maintenance and maintenance.	[a] 1,286	1,920	634	33.02
36	2 and 8	do	do	[a] 1,342	1,947	605	31.07
47	1 and 7	Corn meal	do	[a] 886	3,380	2,494	73.79
36	2 and 8	do	do	[a] 884	3,361	2,477	73.70
47	3 and 7	Alfalfa	Half more than maintenance and maintenance.	[a] 1,425	2,006	581	28.96
36	4 and 8	do	do	[a] 1,425	2,011	586	29.14
47	3 and 7	Corn meal	do	[a] 983	3,531	2,548	72.16
36	4 and 8	do	do	[a] 938	3,472	2,535	72.99

[a] See p. 280 for method of derivation.

TABLE 23.—*Heat increments as computed from differences in the heat production of consecutive planes of nutrition*

Steer No.	Periods compared	Planes of nutrition compared	Heat increment per kilogram of dry matter
			Cals.
47	11 and 5	Fast and half less than maintenance	214
	5 and 7	Half less than maintenance and maintenance	838
	7 and 3	Maintenance and half more than maintenance	1,205
	3 and 1	Half more than maintenance and twice maintenance	966
36	12 and 6	Fast and half less than maintenance	198
	6 and 8	Half less than maintenance and maintenance	815
	8 and 4	Maintenance and half more than maintenance	1,184
	4 and 2	Half more than maintenance and twice maintenance	1,049

The object of the computations represented by Table 18 is to derive values for the heat production of the steers, in different experimental periods and on different planes of nutrition, reflecting the influence of the feed, and being free from the influence of the different live weights of the animals in the several experimental periods.

To this end the maintenance requirement of net energy, in the several periods, was computed from the fasting heat production by modifying this value in proportion to the two-thirds power of the average live weights; and the heat production in the several periods was corrected to a basis of uniform live weight (and maintenance requirement) by adding or subtracting, according to the nature of the correction, the difference between the heat production as observed during fast and the corresponding maintenance quota of net energy, computed as above, for the several feeding periods.

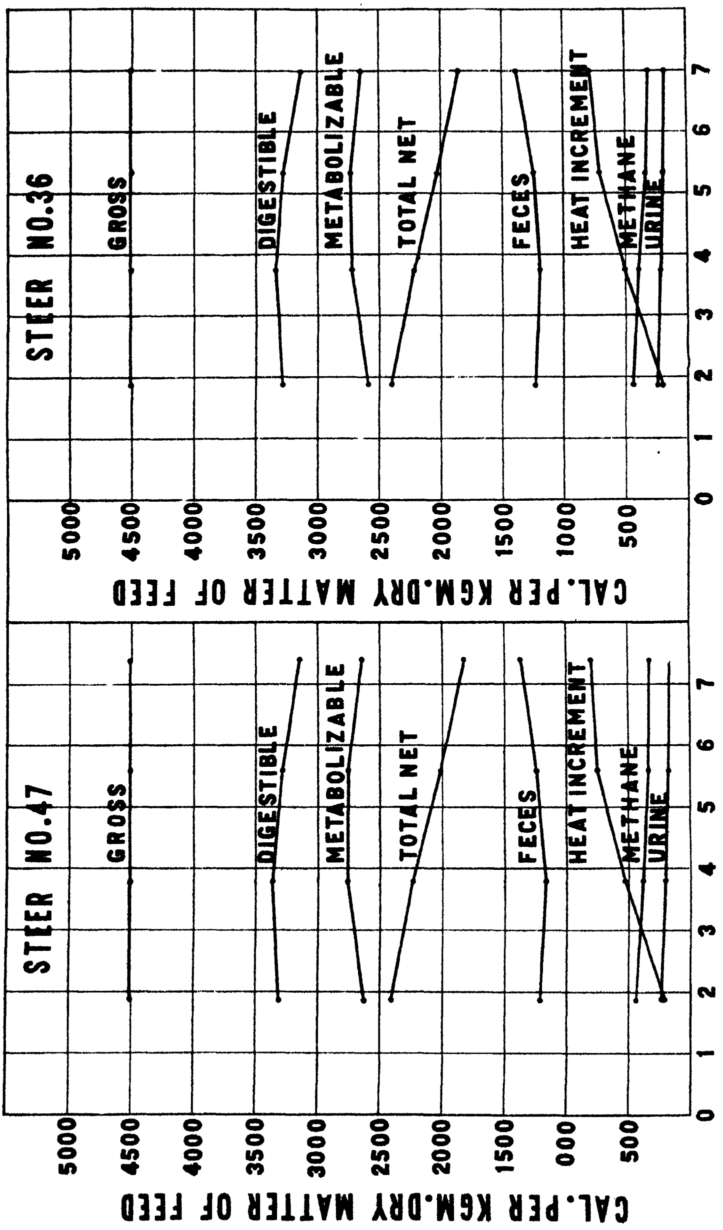

FIG. 1.—Partition of feed energy as influenced by the plane of nutrition of cattle

The uncorrected heat production, as directly observed and as computed by the balance method, and these same values corrected, as above indicated, to a basis of uniform live weight and maintenance requirement, are given in the four columns at the right in Table 18.

In Table 19 the computation is carried a step further, to the total heat increments (energy expenditure of feed utilization) per kilogram of dry matter of feed, in the several periods. These values were obtained by subtracting the heat production of fast from the average of the observed and the computed heat production in each period, corrected, as shown in Table 18, to the basis of uniform live weight and maintenance quota, and dividing by the number of kilograms of dry matter of feed. It should be noted that heat-increment values[4] computed in this manner cover maintenance and production together.

The partition of the gross energy of the ration of corn meal and alfalfa hay, according to the designations—digestible, metabolizable, urine, feces, methane, total heat increment, and total net—as affected by the plane of nutrition, is shown in Table 20 and in Figure 1.

The total net-energy values in this table are for maintenance and body increase together. In view of the fact, therefore, that food energy is used for maintenance and for body increase at different rates of economy, these figures for net energy are mixed values, applying only to the particular planes of nutrition at which the determinations were made and having no general or standard significance.

The difference between these total net-energy values and the separate values for maintenance and for body increase, to be discussed, should be clearly appreciated.

In Table 21 are (1) the net-energy values, for maintenance of the alfalfa hay, of the mixed ration, and of the corn meal, (2) the percentage of utilization of the metabolizable energy of each of the foregoing, and (3) the data leading directly to these values. The net-energy values of the mixed rations for maintenance, with the corresponding heat-increment values, are represented as "Net 1," and "Heat Incr. 1," in Figure 2.

The computation of the net-energy values for maintenance was made by the usual method—the values for the mixed ration and for the alfalfa hay being determined directly by subtracting from the metabolizable-energy value the heat-increment value obtained by comparison of the heat production of maintenance with that of fast; and the value for corn meal being computed by difference, from the net-energy values for the mixed ration and for the hay, by assuming that the hay in the mixed ration has the same net-energy value as that determined for the hay when fed alone.

The utilization of metabolizable energy, as expressed in the last column of Table 21, was computed as the percentage of the metabolizable which is net.

The net-energy values of the mixed rations, for body increase, as recorded in Table 22, were computed by the same general procedure as for maintenance, but with the difference that the heat-increment values were computed from comparisons of the heat production at the higher levels of nutrition with the heat production of maintenance

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[4] The terms "metabolizable energy," "heat increment," and "net energy," according to the usage of this institute, are without definite signification as to quantities of energy of these descriptions; but "metabolizable-energy values," "heat-increment values," and "net-energy values" are the quantities of each *per kilogram of dry matter of feed.*

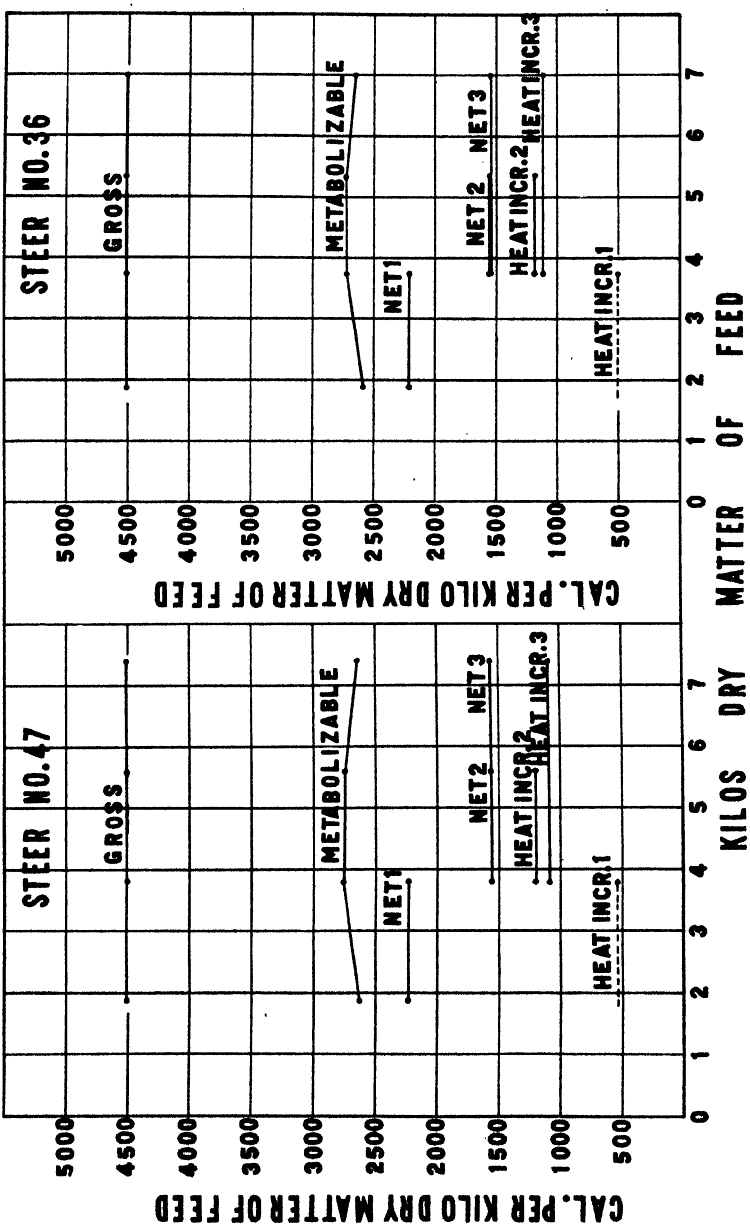

FIG. 2.—Net energy of rations resolved into components for maintenance and for body increase

instead of with that of fast; and the computation of the net-energy values of the individual feeds for body increase was performed by a new procedure, which has been mentioned (p. 257), depending on the assumption that the heat increment and the metabolizable energy of the individual feeds are affected by the plane of nutrition in the same ways and to the same extent as are the same functions of the mixed ration. The net-energy values of the mixed rations for body increase, with the corresponding heat-increment values, are represented, with similar values for maintenance, in Figure 2.

HEAT-INCREMENT, METABOLIZABLE-ENERGY, AND NET-ENERGY VALUES

The heat-increment values for alfalfa hay (Table 22, first column of figures) are decidedly higher than are those relating to corn meal alone, or to the mixed ration, though the metabolizable-energy value of the alfalfa hay is much lower than that of the mixed ration or of the corn meal.

These high heat-increment values of alfalfa hay are due, in large part at least, to the fibrous nature of this feed; but they also direct attention to the effect of its protein constituents in stimulating heat production.

The percentages of utilization of metabolizable energy, as given in the last column, agree almost perfectly for the two steers, and differ in accord with the kind of feed in an apparently consistent manner.

The net energy of the mixed ration and of the individual feeds, for body increase, are given in the third column of figures.

The uppermost four values apply to the mixed ration, the first pair to the interval between maintenance and twice the maintenance requirement, and the second pair to the interval between maintenance and half more than maintenance.

The determinations with steer No. 47 at these two planes of nutrition gave exactly the same value, 1,557 Calories, while the determinations with steer No. 36 also agreed well, being 1,531 and 1,550 Calories, respectively.

There is a distinct difference between the heat-increment values of the ration at these two levels of production, those values involving the larger quantity of feed being the lower. Owing, however, to the fact that the metabolizable-energy values of the ration in the periods of highest feed intake are lower than in the periods of feeding half more than the maintenance requirement, by quantities almost the same as the differences in heat increments, the two sets of net-energy values are almost identical.

The net-energy values of the mixed ration at the two higher planes of nutrition, therefore, seem to be alike.

The second four values represent the plane of nutrition from maintenance to twice the maintenance requirement, the first pair applying to alfalfa hay and the second pair to corn meal; and the third four values represent the plane of nutrition from maintenance to half more than maintenance, the first pair applying to alfalfa and the second to corn meal.

At the higher plane of production (maintenance to twice maintenance) the alfalfa hay has a value somewhat higher than at the lower plane (maintenance to half more than maintenance), which, by the "difference method" of computation, gives to the corn meal at the higher plane a somewhat lower value than at the lower plane.

These differences, however, may represent only experimental error, since the values for the individual feeds are derived by a computation involving not only the difference method, as stated, but also extensive modification of the heat-increment and metabolizable-energy values, as directly determined.

The agreement of the percentages of utilization of metabolizable energy of the mixed ration at the two planes of nutrition is good; and the data for the alfalfa hay as compared with those for the corn meal, at the two planes of nutrition, call attention to the fact that the net-energy value of the hay is lower than that of the grain both because the metabolizable energy of the hay is lower and because its heat increment is higher than that of the grain.

It must be understood that the net-energy values for body increase, which have been discussed, apply not to the ration as a whole but only to that portion of the ration which is in excess of the maintenance requirement.

HEAT PRODUCTION AS AFFECTED BY THE PLANE OF NUTRITION

The heat production as related to the plane of nutrition is exhibited in Figure 3. The data for heat are the averages of the observed and the computed values, corrected to the standard day, and also corrected to correspond to a uniform live weight and maintenance requirement (Table 19); and the data representing the kilograms of dry matter of feed eaten per day may be found in Table 5, or in Table 20.

The order in which the five planes of nutrition were studied is shown by the numerals at the points of observation in the graph, this order having been determined in consideration of a possible carrying over of an influence in relation to the heat production from one period and plane of nutrition to a following period and different plane of nutrition. Accordingly, the animals were first given the largest amount of feed; that is, twice the maintenance requirement, followed in order by half more than maintenance, half less than maintenance, maintenance, and fast.

In this study it was considered especially important to reach a correct value for fasting, an object rendered difficult by the fact that the heat production of fast, however determined, is not an absolute value, but is somewhat affected by the conditions of determination, especially in regard to the duration of fast and to the previous plane of nutrition. The authors have proceeded, therefore, with the understanding that the methods of determination of the fasting katabolism, as a measure of the maintenance requirement of net energy, must be rigidly standardized; and the sequence of treatments was so arranged, as a routine procedure, that fasting periods should always follow periods at the maintenance level of nutrition. Careful attention has been given the control of this measurement, and the authors feel that the maximum aggregate error of work affecting the values used in this study can not be of such magnitude as would materially alter the curve of heat production in relation to plane of nutrition.

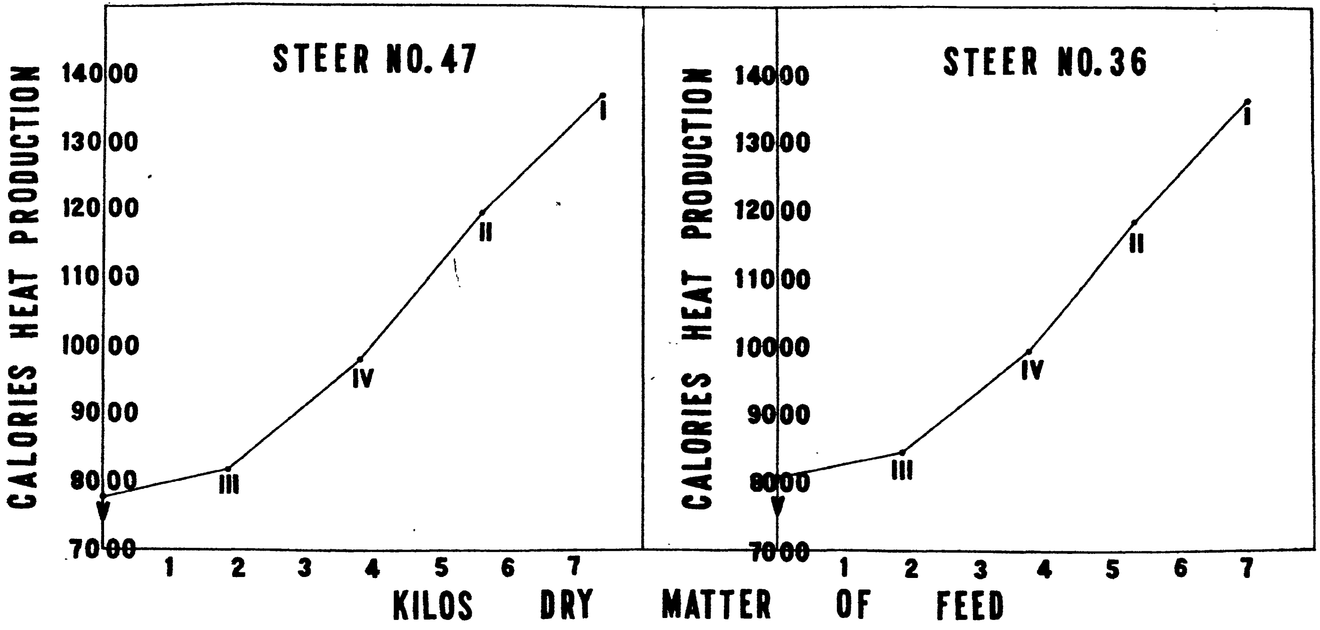

FIG. 3.—Heat production as affected by the plane of nutrition of cattle

A study of Figure 3 shows, at once, a remarkable concordance of the curves representing the heat production of the two steers at the several planes of nutrition. This agreement indicates that the experimental routine was well in hand, and especially that the validity of the results, for comparative purposes, was not seriously vitiated by effects of any uncontrolled and unrecorded physical activity of the experimental subjects.

The heat production increased but little from fast (V) to the plane of half of the maintenance requirement (III), and increased much more rapidly from the latter plane of nutrition to the maintenance level (IV); but the entire rise in metabolism from the fasting to the maintenance level was at a distinctly lower rate than was the fairly uniform increase from the level of maintenance (IV) to that of twice maintenance (I).

Several factors appear to contribute to the determination of the character of this curve representing the relation of the heat production to the plane of nutrition.

In harmony with the point of view of this paper the maintenance requirement of net energy is a constant measured by the heat production of fast, which makes no contribution to the curve of heat production except as its amount, or height, fixes the lower point of the curve.

In this light the curve representing the heat production at increasing rates of food consumption may be explained as resulting from the following influences: (1) The stimulation of the heat production through the specific dynamic effect of protein and the so-called "metabolism of plethora" (of fat and carbohydrate), as Lusk uses this term; (2) the energy expenditure of synthesis of body nutrients above maintenance (fat, from carbohydrate); and (3) the decreased metabolizability of the food at the higher planes of nutrition.

The slight divergence of the curve toward the horizontal at the highest as compared with the next lower plane of nutrition is apparently due in part at least to this last condition; that is, to the fact that the amounts of food available for the use of the animal are not quite proportional to the amounts of food consumed.

From another point of view, on hypothetical grounds not in harmony with the fundamental conception of the observed heat production of fast as the measure of the maintenance quota of net energy, an important factor in the determination of this curve of heat production would be an assumed specific dynamic effect of body nutrients katabolized below maintenance.

Thus, differences in the specific dynamic effect of circulating metabolites, due to the decreasing amount of body nutrients and increasing amount of food nutrients katabolized between fast and maintenance, and to the changing proportions of fat, protein, and carbohydrate in the body nutrients katabolized between fast and maintenance, would contribute prominently to the composition of this curve.

In accord with this hypothesis the maintenance quota of net energy would be conceived of as the heat production during a status of fast in which the energy requirement of the animal would be rendered available without waste of heat, that is, without energy expense of utilization; this heat production, therefore, being a true measure of the energy requirement. This hypothetical minimum maintenance requirement, then, would be a lower value than the actual fasting katabolism by an amount assumed to represent an energy expense of utilization, or a specific dynamic effect, of body nutrients oxidized during fast.

The hypothetical minimum maintenance requirement of net energy above suggested is not now a practicable or a desirable base value for use in studies of the energy of feeds, however, (1) since it can not be measured directly; (2) since no method has been devised for computing it with satisfactory invariability; and (3) since this conception would necessitate the abandonment of the directly determined and universally accepted measure of the maintenance quota in favor of a computed datum the physiological significance of which has not been established.

Also, if there were an opportunity to choose between these two measures of net-energy requirement of maintenance, it would not be quite as between a correct and an incorrect value; because although the lower figure would give a lower net-energy value, and the higher, a higher net-energy value, of the feed, the quantity of feed required for maintenance would be reckoned as the same in either case.

It may be helpful in appreciating the significance of the two standards of measurement of the maintenance requirement of net energy in relation to the curve of heat production, to consider that they differ as though between regarding this curve from its upper end, from which point of view the striking fact is the increased heat production in relation to feed eaten, below maintenance; and from its lower end, from which point of view there is a marked increase in heat production in relation to feed eaten, above maintenance.

The final decision between these two points of view, that is, as to whether the curvature of the line representing the heat production between the levels of fast and maintenance expresses a factor of waste in the heat produced from the body substance katabolized, or a variation in the energy cost of utilization of food, must await the quantitative analysis of the heat production of fast, that is, the measurement of the factors contributing thereto.

Whatever the point of view from which the curve of heat production is regarded, and whatever the value employed as the maintenance requirement of net energy, it is well to realize that the energy cost of utilization of food for maintenance is measured as the difference between the maintenance requirement of net energy (however measured) and the heat production at the maintenance level; the net-energy value of the food, for maintenance, and the relative efficiency of utilization of food for maintenance as compared with body increase, therefore, are directly affected by the magnitude of the figure (directly observed fasting heat production, or lower, hypothetical, minimum heat production) accepted as the measure of the maintenance quota of net energy.

During fast, body tissue is used as a source of energy for the vital activities of the animal, and, according to present usages, the maintenance requirement of net energy is measured by the metabolizable energy of the body tissue katabolized.

If, however, a sufficient quantity of feed is given to prevent any loss of body tissue, the feed instead of the body tissue is used as a source of energy for the vital activities of the animal, that is, for maintenance; and the net-energy value of the feed, the determination of which present usage involves the comparison of the heat production of fast and maintenance, is its value for the prevention of body loss, that is, for maintenance.

Above the maintenance level there is no replacement of body nutrients by feed nutrients, as there is below maintenance, and the amount by which the heat production is greater than that at maintenance is a direct measure of the cost of utilization of feed energy for the production of body increase.

The curves, as well as the heat-increment values in Tables 21 and 22, show a distinct difference between the terms of the two uses of feed energy, and that it is utilized more efficiently to prevent loss than to produce increase of body tissue.

It is impossible to assume, therefore, as was done in the publications of this institute up to 1925, except on the basis of the hypothesis on page 285, which is not definitely advocated, that the rate of utilization of feed energy is the same above as it is below the maintenance level of nutrition.

The most practicable method of recognition of these differences is through the determination of separate net-energy values of feeds for maintenance and for body increase, as recorded in Tables 21 and 22, and as discussed in a later section of this paper.

In this connection mention should be made of the rate of utilization of feed energy for milk production, which was shown in a recent paper (*9*) from this institute to be essentially the same as that at which it is utilized for maintenance. This may be understood as consistent if the milk is considered as potential but unorganized body substance, and the nutritive requirement of milk production as an increase in the maintenance requirement of the body, since during milk production and liberal feeding the body itself remains approximately in energy equilibrium.

A consequence of the facts as exhibited with reference to the relationship between the heat production and the plane of nutrition, between fast and maintenance, which should be considered in the determination of the specific dynamic effects of foods, or of nutrient compounds, at planes of nutrition between these levels, in comparison with fast, is that such dynamic effects must differ prominently with the planes of nutrition at which the observations of heat production are made. In this light it appears that comparable determinations of specific dynamic effects of nutrients can be made only at the same plane of nutrition.

Figure 4 is presented in an effort to make clearer the relations between the heat production, the gross energy, the metabolizable energy, the maintenance quota, the heat increment, the body increase (above maintenance), and the body loss (below maintenance) as affected by the plane of nutrition. For this purpose the actual results obtained with steer No. 47 have been plotted.

The area *BAK* covers the gross energy of the rations. The very slight departures of the line *BA* from a straight line are the result of slight differences in the composition of the same kinds of feed used in the several experimental periods.

The area *BAC* covers the energy of the excreta; and *CAK*, the metabolizable energy of the rations.

The point *J* represents the heat production of fast, which is the measure of the maintenance requirement of net energy; and the line *JE* expresses the assumption that the maintenance requirement of net energy is a constant at all planes of nutrition.

The line *JD* (heat) crosses the line *AC* (metabolizable energy) at *F*, the point of energy equilibrium.

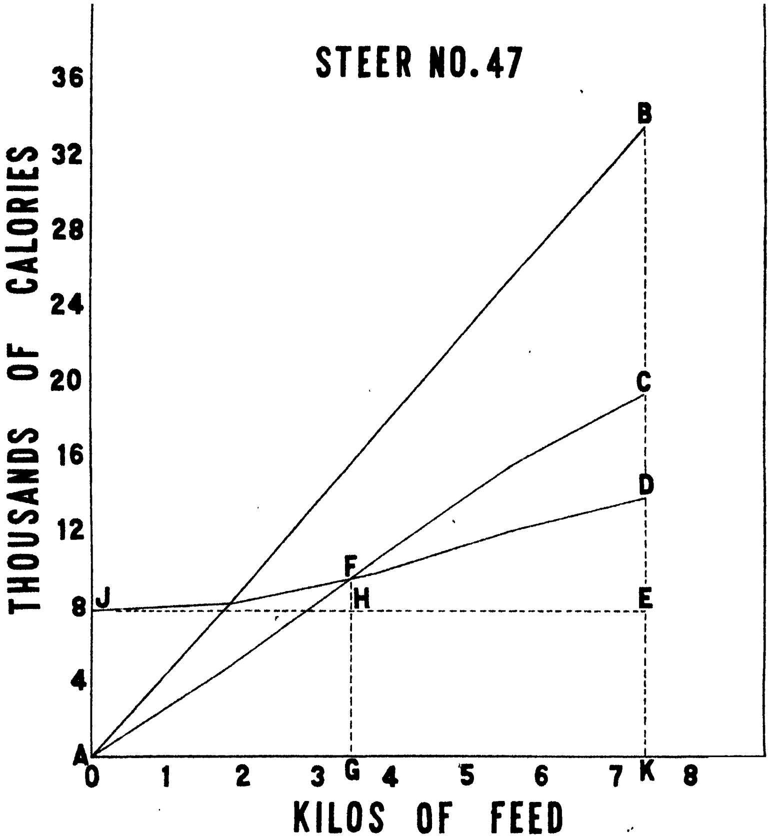

FIG. 4.—Relations of metabolizable energy, heat production, and maintenance quota of net energy to the gross energy of the feed

The area *DJE*, the difference between the heat production and the maintenance requirement of net energy, represents the heat increment or energy expenditure of feed utilization.

Since the heat increment is a part of the metabolizable energy it would be represented in this natural relation with the line *JD* in the line *AC*, the point *E* falling near the point D.

The area *FJH* expresses the heat increment below maintenance; and *DFHE*, the heat increment above maintenance.

The area *FAG* represents the metabolizable energy of the feed, and also the heat produced from the feed, below maintenance; *JFA*, the heat produced at the expense of body substance, below maintenance; *CFD*, the energy of the body increase, above maintenance; while the net energy of the rations would be represented as the difference between the metabolizable energy, *CAK*, and the heat increment, *DJE*.

As results of direct experimental observations the metabolizable energy, the heat increment, the body gain, and the body loss are all curvilinear functions.

It will be impossible to explain these observations in fundamental ways until the quantitative analysis of the fasting katabolism, and of the heat increment as affected by the plane of nutrition, have been accomplished, and until the reasons for the relations of the digestibility and the metabolizability of the food to the plane of nutrition are understood.

DIGESTIBILITY OF THE FEED AND PARTITION OF THE GROSS ENERGY IN RELATION TO THE PLANE OF NUTRITION

In the exhibit of the disposition of the feed energy, in Calories per kilogram of dry matter of the ration of corn meal and alfalfa hay as affected by the plane of nutrition, set forth in Table 20 and in Figure 1, the points of observation were the same as those indicated in Figure 3; and, as in Figure 3, the agreement between the data representing the metabolism of the two animals is so remarkably close that all general conclusions made with reference to one apply to the other.

The gross energy of the feed at the four planes of observation was, of course, the same, as shown by the horizontal line in Figure 1.

The energy of the digestible nutrients rose very slightly from submaintenance to maintenance, with both steers, this increase being due to a greater digestibility of the crude fiber of the ration at the maintenance than at the submaintenance level. (See Table 2.)

The fall in the curve of energy of digestible nutrients, from the maintenance level to half more than maintenance, and a second fall from the level of half more than maintenance to the highest level, was due mainly to decreased digestibility of carbohydrates and protein.

The significance of data representing digestible nutriment of rations for ruminants differs somewhat from that of such data for other animals, since the method used for computing digestibility reckons the methane produced by the characteristic fermentation in the ruminant paunch as digestible, which, of course, it is not—in a physiological sense.

The metabolizable energy is that portion of the feed energy which can be converted by the animal into heat, or into body products other than excreta. It is the gross energy of the feed minus the energy of the urine, feces, and methane. Since, in this study, the methane and urine curves are nearly straight lines, the proportions of the feed energy so represented diminishing slightly with rise in the plane of nutrition, the curve of metabolizable energy closely follows that of digestible energy, the difference between the two diminishing slightly with increase in feed.

The curve representing the energy of the feces is, necessarily, exactly the reverse of that representing the energy of the digestible nutrients.

The potential energy of the methane produced by cattle from various feeds commonly varies from about 6 to about 11 per cent of the gross energy; and in the experiment here discussed varied from 6.4 to 9.8 per cent of the gross energy. If the heat of the fermentation which liberates the methane is, as reckoned by Krogh (*18*), 50 Calories per gram molecule of CH_4, this would be equal to about one-fourth of the potential energy of the methane, and therefore to 17.5 per cent of the heat increment of the feed between fast and maintenance in this study.

The curve of methane production, indicating a slight decrease per kilogram of feed, with increase in the plane of nutrition, is in harmony with observations on this point at this institute during many years and is probably the result of less protracted stay of the larger than of the smaller intake of feed in the paunch, a decrease in feed being followed by a smaller proportionate decrease in paunch content.

The line representing the energy of the urine is nearly straight and nearly horizontal, and would be expected to conform perfectly to both conditions if the correction of the urinary energy, given in Table 4, were exact. The slight divergence of this curve from the expected straight and horizontal line may be due to inaccuracy of the factor used in making the above-mentioned correction, or to some unknown cause.

Figure 1 shows that the total heat increment per unit of feed (computed in each case with reference to the fasting heat production) rose significantly with rise in the plane of nutrition, but the rate of increase in heat increment was such as to be expressed not by a straight, ascending line, but by a curve diverging downward from such a line. Obviously, these widely differing heat increments suggest a different net-energy value at each plane of nutrition; but a way out of the dilemma thus presented is shown in the discussion of net-energy values for maintenance and for body increase.

Finally, the total net-energy values of the rations decreased with increase in the plane of nutrition as a practically rectilinear function of the quantity of the feed; and, as has been stated in connection with the discussions of the heat increments, these are in a sense mixed values, each having a different significance. Reading from left to right, the first represents the highly efficient utilization of feed energy to prevent body loss at a very low plane of nutrition (half of the maintenance requirement); the second represents the less efficient utilization of feed energy at the plane of energy equilibrium; and the third and fourth represent the same value for net energy for maintenance plus different quantities of net energy for body increase, the latter being used at a lower rate of efficiency.

The net energy of the rations resolved into components for maintenance and for body increase is indicated in Figure 2 and will be discussed in the following section.

SIGNIFICANCE OF THE RESULTS OF THIS STUDY IN RELATION TO THE PROBLEM OF METHODS OF DETERMINATION OF THE NET-ENERGY VALUES OF FEEDS

The influence of the plane of nutrition on the problem of methods of determination of net energy will be considered in relation (1) to mixed rations, (2) to individual feeds, (3) to maintenance, and (4) to production.

It has long been a matter of common knowledge and observation that the digestibility of feeds is affected by their combination in rations in such sense that the digestibility of the components of a ration may be greater, or, in some cases, less, than if the feeds are eaten separately; and the net energy also is directly affected through this influence, since only digested nutrients contribute to the net energy.

In this matter the situation of especial interest is the reciprocal effect of grain and roughage, since the digestibility of the grain by ruminants is always determined by difference between results from mixed grain and hay rations and rations of hay alone, for the reason that, inasmuch as ruminants do not ruminate when fed grain alone, it is considered that grain would not be normally metabolized under such conditions.

In the determination of the digestibility of grain by ruminants, therefore, the entire effect of combining the grain and roughage is assigned to the grain. This is obviously illogical, and the error seems inevitable; but the situation is not so unsatisfactory as it at first appears, because since grain is fed to ruminants only in combination with roughage, the digestibility determined by difference, as above indicated, while perhaps actually in error, does apply to the general conditions under which grain is fed.

This aspect of the problem, therefore, may be dismissed from further consideration for the present; and inasmuch as the energy of the urine and of the methane produced from the mixed ration fed in these experiments is shown in Figure 1 to be in both cases an essentially rectilinear function of the dry matter of the feed, and further since these two factors of energy outgo are not influenced in important ways by the combination of feeds, they likewise require no further consideration in this relation.

Regarding the heat increment, however, the problem is complicated by the facts that this is not a rectilinear function of the dry matter of the feed; that we do not know that the heat increment of combined feeds is under all conditions the sum of their separate heat increments; or that the heat increments of all components of a ration are affected by the plane of nutrition in proportion to such effect on the heat increment of the ration as a whole.

In these experiments it so happens that the feces curve, combined with the heat-increment curve, would result in a practically straight line. Then, since the urine and methane curves are nearly straight, the curve representing the combination of all these losses and expenses of feed utilization must be a nearly straight line, as obviously is also the gross energy, and therefore the total net energy, since the net is the difference between the gross energy and the sum of the losses and expenses of utilization.

The net-energy values of the ration for maintenance and for the two rates of supermaintenance feeding, computed by the method given on page 280, and as indicated as Net 1, Net 2, and Net 3, in Figure 2, are, as a result of the method of derivation, necessarily rectilinear functions; also the net-energy value of the ration at the two planes of supermaintenance feeding (Table 22) were, with steer No. 47, identical, and with steer No. 36, essentially alike.

The net-energy values represented in this graph were computed by the subtraction of the corresponding heat-increment values, as there shown, from the metabolizable energy.

In regard to the net energy of the ration as a whole, either for maintenance or production, then, the situation in the light of the results of these experiments seems satisfactory. The evidence at hand does not afford the basis for final judgment, however, as to the practicability of determining net-energy values of individual feeds, particularly at the production levels of nutrition, since, as indicated above, we are in need of further information as to the effects of feed combination on the dynamic potencies of the individual feeds involved, and especially since in the event that the dynamic effect of a ration of hay and grain together should be found not to be the sum of the dynamic effects of these components, and further should it be shown that the influences of the plane of nutrition on the heat increments of each component of a ration is not proportional to such influence on the ration as a whole, such differences would have to be assigned, though improperly, to the grain alone—which might leave the dependent net-energy values of individual feeds quite without distinctive significance.

TABLE 24.—*Distribution of gross energy of feeds*

	Experiment No.	Gross energy of dry matter	Losses of chemical energy in—			Percentage of nutriment that is digestible	Heat-increment for maintenance	Net energy for maintenance	Percentage of energy of digestible nutriment that is net
			Feces	Urine	Methane				
		Cals.	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*	
Corn silage	237	4,606	31.7	3.6	8.5	68.3	10.7	45.5	66.7
Soy-bean hay	237	4,524	39.9	6.4	7.5	60.1	12.9	33.3	....
Do	237	4,555	38.8	5.8	7.6	61.2	10.7	37.1	....
Average, soy-bean hay	237	4,540	39.4	6.1	7.6	60.6	11.8	35.2	58.1
Alfalfa hay	237	4,522	47.4	5.6	5.7	52.6	13.2	28.1	....
Do	237	4,615	47.8	5.4	6.0	52.2	12.0	28.8	....
Do	238	4,479	43.5	5.1	6.5	56.5	14.0	30.9	....
Do	238	4,479	43.7	5.1	6.4	56.3	13.6	31.1	....
Average, alfalfa hay	238	4,524	45.6	5.3	6.2	54.4	13.2	29.7	54.6
Oats, ground	237	4,752	28.8	3.8	10.1	71.2	10.6	46.8	....
Do	237	4,713	22.9	4.4	9.8	77.1	9.3	52.5	....
Average, oats, ground	237	4,733	25.9	4.1	10.0	74.1	10.0	49.7	67.1
Corn meal	238	4,558	7.9	3.9	10.4	92.1	9.5	68.3	....
Do	238	4,558	8.8	4.3	11.0	91.2	8.8	67.1	....
Average, corn meal	238	4,558	8.4	4.1	10.7	91.6	9.2	67.7	73.9

It was the expectation of the authors, at a comparatively recent date, to determine net-energy values of individual feeds for body increase from such values for maintenance by multiplying the latter by a previously established ratio of net energy for body increase to net energy for maintenance; but it is now apparent that such a ratio obtained with a mixed ration would not correctly apply to its individual components (1) because, as shown in Table 24, the heat-increment values of different feeding stuffs are not proportional to their metabolizable-energy values, and (2) because, as shown in

Figure 4, the metabolizable-energy value and the total heat-increment value, from which the net energy is computed, are not affected alike by the plane of nutrition.

Such a computation as above suggested would lead to the apparently unreasonable assumption that the effect of the plane of nutrition on the heat increment of the individual components of a ration is such as materially to alter their relation to each other. For example, in this experiment, whereas the heat-increment value for maintenance, of corn meal, has been found to be less than the heat-increment value of alfalfa hay, the heat-increment value for body increase, of corn meal, on the basis of the above assumption, would be greater than the corresponding value for alfalfa hay—as illustrated below, with data applying to the maintenance and the twice-maintenance periods, with steer No. 47.

Net-energy value of mixed ration for maintenance = 2,241 Cals.
Net-energy value of mixed ration for body increase = 1,557 Cals.
Ratio of net for body increase to net for maintenance = 0.695:1.
Heat-increment value of alfalfa hay for maintenance = 628 Cals.
Heat-increment value of corn meal for maintenance = 433 Cals.
Net-energy value of alfalfa hay for maintenance = 1,385 Cals.
Net-energy value of corn meal for maintenance = 3,111 Cals.
Net-energy value of alfalfa hay for body increase = 1,385 (net, maintenance) × 0.695 (factor) = 963 Cals.
Net-energy value of corn meal for body increase = 3,111 (net, maintenance) × 0.695 (factor) = 2,162 Cals.
Metabolizable-energy value of alfalfa hay for body increase = 2,013 Cals.
Metabolizable-energy value of corn meal for body increase = 3,544 Cals.
Heat-increment value of alfalfa hay for body increase = 2,013 (metabolizable) − 963 (net) = 1,050 Cals.
Heat-increment value of corn meal for body increase = 3,544 (metabolizable) − 2162 (net) = 1,382 Cals.

This demonstration of the fact that the metabolizable-energy values and the heat-increment values of the components of a ration, from which the net-energy values of these components are directly computed, are not affected alike by the plane of nutrition, leads naturally to the question of the causes of this situation.

These seem to be the effects (1) of the "difference method" of computation of the above-mentioned values of the components, and (2) of the fact, exhibited in Figure 1, that while the heat-increment and metabolizable-energy curves for the mixed ration are much alike, the heat-increment curve falls with decrease in the plane of nutrition, while the metabolizable-energy curve remains, in general, about on a level, these values of the ration as a whole, therefore, being differently affected by the plane of nutrition.

What change of basis of computation of these curves would tend to make them more nearly alike? Obviously, a lower value for the fasting metabolism—such, for instance, as the hypothetical value suggested on page 285. As has been stated, however, no satisfactory method for the derivation of such a value has been proposed.

The assumption, which is made the basis for computing the net-energy values of individual feeds for body increase in this paper (Table 22), is that the relation of the heat increments of the individual feeds to each other is not changed by the plane of nutrition; but that the magnitude of the heat increments of these individual feeds is affected in the same proportions as is the heat increment of the mixed ration composed of these feeds; and that the metabolizable-energy values of the individual feeds are also affected by the plane of nutrition in the same proportion as is the metabolizable energy of the mixed ration.

Two sets of such values were determined with each animal, representing the increase (1) from maintenance to half more than maintenance, and (2) from maintenance to twice the maintenance level of nutrition.

The heat-increment values used in computing these net-energy values of the individual feeds for body increase were derived from the corresponding heat-increment values for maintenance by increasing the latter in proportion as the heat-increment values of the mixed rations for body increase exceeded the corresponding values for maintenance; and the metabolizable-energy values of the single feeds for body increase were derived from the corresponding values for maintenance by decreasing the latter in proportion as the metabolizable-energy value of the mixed ration for body increase was less than the corresponding value for maintenance.

This new method of computation of net-energy values of individual feeds for body increase is not definitely and finally recommended, but is proposed for study, as one to which the authors have not yet found fatally disqualifying objections.

A question is raised as to the validity of such a computation by the character of the results obtained in these experiments. Thus, the ratio of the net-energy values of the mixed ration, and of the individual feeds for body increase (twice the maintenance) to the corresponding values for maintenance, would be as follows:

Steer No. 47, alfalfa hay, 634 : 1,385=0.465
No. 36, alfalfa hay, 605 : 1,395= .434
No. 47, corn meal, 2,494 : 3,111= .802
No. 36, corn meal, 2,477 : 3,059= .810
No. 47, mixed ration, 1,557 : 2,241= .695
No. 36, mixed ration, 1,531 : 2,218= .690

The net-energy values (for body increase) of the corn meal are represented as being about four times as great as those of the alfalfa hay; and the ratio of the net-energy value of the corn meal for maintenance to the same for production is very much higher than is the ratio between the corresponding values for alfalfa hay. These values may be correct, aside from the errors incident to the use of the "difference method" involved in the computation of the net-energy value of the corn, but are surprising, and require confirmation.

The advantage of separating the total net energy of a ration into net energy for maintenance and net energy for body increase lies in the fact that by so doing is revealed the proportionality which exists between the different nutritive energy requirements of these activities, thus providing a convenient basis for computing the total feed requirement for a particular rate of body increase.

Table 23 is presented as an exhibit of the diversity of heat increments which would result from the comparison of the heat production at the different planes of nutrition, variously paired; that is, representing different parts of the curve of heat production.

This diversity of heat increments results from the fact that the heat production at various levels of nutrition is not a rectilinear function of the feed eaten. If the heat production were a rectilinear function of the feed, all these increments would be alike.

The character of these values emphasizes the necessity for the conventional standardization of the methods of determination of net-energy values.

A situation of general significance, applying not only to the results of the present study but to all determinations of net-energy value made by the methods followed at this institute, is that such values of individual feeds, either for maintenance or for body increase, but especially the latter, are not characterized by a consistency commensurate with the extreme particularity and refinement of the general method of experimentation.

This is a result of the fact previously observed (pp. 253 and 254) that the method of this determination depends in part only on physical and chemical measurements of great accuracy, but in part also on conventional assumptions and procedures which seem to be necessary for the derivation of simple and convenient measures of nutritive value, for guidance in practical feeding, but which assume a kind and degree of physiological orderliness which in reality does not exist.

The refined methods of work followed in this study are fully justified, however, by the fact that the objects of the experiments are not net-energy values alone, but embrace also the elucidation of principles of nutrition in general, for which purpose the most refined methods known fall short of ultimate if not of present requirements.

Another fact of general significance in this relation is that the whole method of experimentation in the determination of net-energy values of feeds presupposes that all essential nutrients other than those used for energy production are present in optimum quantities in all rations compared, so that nutritive energy values are unaffected by deficiencies of other than energy-producing nutrients.

It is the belief of the writers that this assumption, as relating to the energy studies of this institute, does not involve serious error, but it is their intention to undertake, at an early date, studies for the specific purpose of learning the magnitude of any such errors as may result from the suggested cause.

In regard to both of the above considerations it is important to understand that net-energy determinations may be of great value even though not absolutely accurate, since they are presumably much more nearly accurate than are the digestible nutrient values on which the most commonly used American feeding standards are based.

To illustrate certain facts in this relation the writers present in Table 24 an exhibit of a separation of the gross energy of several feeds into component fractions, as determined in recent experiments at this institute, leading finally, in the last column, to a statement of the percentage of the energy of the digestible nutriment of each feed which is net energy.

Obviously, if digestibility were of the same significance as a measure of energy value as is net energy (assuming that net energy is a true measure of energy value) the percentage of the energy of the digestible nutriment which is net would be the same, regardless of the nature of the feed.

These figures show, however, that this percentage varies from 54.6 for alfalfa hay to 73.9 for corn meal, the difference between these percentages representing the inaccuracy of digestible nutriment as a measure of energy value, and suggesting also that the most important advantage of net-energy values as compared with digestible nutrient values is that the former differentiate more nearly correctly than do the latter between the energy values of grains as compared with roughages; further, net energy serves to measure nutritive value in terms of the product, thus indicating how much of the product will result from a given quantity of feed, while digestible nutrients are not characterized by any such significance.

SIGNIFICANCE OF THE RESULTS OF THIS STUDY IN RELATION TO PUBLISHED NUTRITIVE EVALUATIONS OF FEEDS

The relationship of the heat production to the plane of nutrition as revealed by this investigation, makes it impossible to assume, as was done in all papers from this institute which reported net-energy values of feeds, prior to and including a revision in 1925 (*10*) of the previously published net-energy determinations, that the efficiency of utilization of feed energy is the same above as it is below the maintenance level of nutrition.

The first papers in the light of the new understanding were one in 1926 (*9*), in which the different rate of utilization of the energy of feed for maintenance as compared with production of body increase was reported; and a second (*8*) in 1927, in which certain net-energy values of feeds for maintenance were published, though obviously such values are exactly true only at the point of energy equilibrium.

In all papers published from this institute prior to those above mentioned, which dealt with net-energy values, such diversity of heat increments as is exhibited in Table 23 was treated as an expression of experimental error—an assumption which has appeared less and less probably true from year to year, as the methods of experimentation involved in this work have been rigorously scrutinized and have been improved in many details. In the light of the results of the present study, therefore, such diverse heat increments as referred to might all be correct.

In so far as the net-energy values published by Armsby and Fries (*3*) and revised by Forbes and Kriss (*10*) are based on both submaintenance and supermaintenance feeding, they must be regarded, in the light of the present findings, as mixed values, but approximating more closely values for body increase than values for maintenance.

The net-energy values of feeds for cattle published by Armsby in the appendix of his book "The Nutrition of Farm Animals" (*2*) are based on the average composition and digestibility of American feeding stuffs, as compiled by Henry and Morrison (*14*), and on values for metabolizable energy and heat increment derived from the work of Armsby and Fries (*3*) and of Kellner and Köhler (*16*).

Armsby and Fries's experiments included both submaintenance and supermaintenance periods; while Kellner and Köhler's experiments (*2*, *16*, *17*), which contributed much to the derivation of the tables of values in Armsby's book, involve mostly supermaintenance periods.

Kellner and Köhler's starch values are, therefore, at least in the main, actual production values, and to this extent the significance of these values is unaltered by the present findings.

Armsby regarded the net-energy values in his above-mentioned book as applying both to maintenance and to body increase. From the nature of the computation by which these values were derived they must be regarded as representing more closely values for body increase than values for maintenance. In general it may be assumed that such of Armsby's values as are based on Kellner and Köhler's work represent production values, while values based on the experiments of Armsby and Fries, especially values for roughages, have been affected by results of submaintenance feeding.

Møllgaard (*19*, *20*) has determined net-energy values of feeds by procedures in general harmony with the understanding of Kellner and of Armsby; and inasmuch as his basal rations were sufficient to cover the maintenance requirement of the experimental subjects, his determinations of net energy for production seem not to have been vitiated, as were some of Armsby's, by the use of data from submaintenance periods.

Møllgaard states energy values, for any nutritive purpose, in terms of net energy for increase of body substance. This procedure may serve, temporarily, as a basis for the computation of feed requirements, for practical purposes, but, in the light of the results of the present study, is imperfect in that it implies a constant relation between net-energy values of a feed for the different functions of maintenance, body increase, and milk production, irrespective of the kind of feed (see p. 293).

SUMMARY

A series of experiments was conducted with steers as subjects, both by the methods of direct and indirect respiration calorimetry, for the study of the energy metabolism in relation to the plane of nutrition, and related problems.

Five planes of nutrition were studied with each of two steers. As indicated by the quantity of feed given, these planes were (1) fast, (2) half of the maintenance requirement, (3) maintenance (energy equilibrium), (4) half more than maintenance, and (5) twice the maintenance requirement.

In the feeding periods the rations were composed of corn meal and alfalfa hay, in equal weights of dry substance, except in one period, with each steer, in which the ration was alfalfa hay alone.

An experimental unit, except as modified in the fasting periods, normally consisted of a 28-day interval, embracing a 10-day preliminary period on the experimental feeding treatment to follow, and an 18-day digestion period, the last three days of the 18 also constituting a continuous, respiration-calorimetric experiment.

Among the new experimental procedures employed in this study were (1) the adoption of the area of the removed hide as the measure of the surface area of the animal, (2) the use of the respiratory quotient, and (3) of the amount of the feed residues in the alimentary tract, as well as the heat production, as usual, as criteria in the standardization of the conditions for the determination of the fasting katabolism as the measure of the maintenance quota of net energy, (4) the correction of the heat production to correspond to a uniform live-weight and maintenance requirement of net energy, in the comparison of the heat production at different planes of nutrition, (5) a new method of computing net-energy values of individual feeds for body increase, based on the assumption that the heat-increment and metabolizable-energy values of the components of a ration are affected by the plane of nutrition in the same ways and degrees as are the heat increment and metabolizable energy of the ration as a whole, and (6) the use of a new procedure in computing the heat production to the standard day as to standing and lying.

The subjects were two Aberdeen-Angus steers, between 25 and 33 months of age, and throughout the study the results with the two steers agreed phenomenally well.

The heat production increased but little from fast to the plane of half of the maintenance requirement, and increased much more rapidly from the latter plane of nutrition to the maintenance level; but the entire rise in metabolism from the fasting to the maintenance level was at a distinctly lower rate than was the fairly uniform increase from the level of maintenance to that of twice the maintenance requirement.

The relation between the heat production and the food consumption, above maintenance, therefore, is expressed by a line of slight curvature and, below maintenance, by a pronounced curve.

The several factors which determined the curve of heat production in relation to the plane of nutrition seem to be (1) the amount of the observed heat production of fast (the base value), (2) the energy cost of utilization of food, (3) the difference in the energy cost of utilization of food for body increase as compared with the utilization of the same for the prevention of body loss, especially as affected by the specific dynamic effect of protein, by the "metabolism of plethora" (of fat and carbohydrate), and by the energy cost of synthesis of fat, and (4) the influence of the incompleteness of digestibility and metabolizability of the food on the proportions of the same which actually contribute to the heat production.

Another point of view, not in harmony with the accepted idea of the observed heat production of fast as the measure of the maintenance requirement of net energy, but depending on the hypothesis of a lower value for this datum, differing from the above by the amount of an assumed specific dynamic effect, or energy expense of utilization, of body nutrients oxidized during fast, would go far toward affording an explanation of this curve. Thus, in accord with this principle, this curve would be influenced by the dynamic effects of the differing proportions of food nutrients to body nutrients, and by the differing proportions of carbohydrate, fat, and protein of body nutrients, katabolized at planes of nutrition between fast and maintenance.

The curves of distribution of feed energy per kilogram of dry matter between feces, urine, methane, and heat increment, and digestible, metabolizable, and net energy, as affected by the plane of nutrition, were plotted.

From the plane of half of the maintenance to that of twice the maintenance requirement these curves were characterized as follows:

Digestible energy first rose slightly, on account of increased digestion of crude fiber, and then decreased, at an increasing rate, as a result of lowered digestion of carbohydrate and protein.

The curve of energy of the feces, naturally, was the reverse of that of digestible energy.

The curves of methane and urine energy were nearly straight lines, falling slightly with rise in plane of nutrition.

The curve of total heat increment (computed with reference to the fasting katabolism) rose at each period of observation, but each time at a decreased rate of rise.

The curve of total net energy (for maintenance and body increase together) was a practically straight line, falling rapidly with rise in plane of nutrition.

The curve of total heat increment was separated into components for maintenance and for body increase, that for body increase (computed with reference to the heat production at maintenance) being much higher than that for maintenance (computed with reference to the fasting katabolism).

From these heat increments and the corresponding metabolizable-energy values different net-energy values of the feeds for maintenance and for body increase were computed.

To such extent as the earlier net-energy values determined at this institute were affected by being derived in part each from the results of submaintenance feeding periods they are of mixed significance.

The net-energy values of the feeds for maintenance, with the two steers, were, for alfalfa hay, 1,385 and 1,395 Calories, for corn meal, 3,111 and 3,059 Calories, and for the mixed ration, 2,241 and 2,218 Calories, per kilo dry matter.

The corresponding net-energy values for body increase, at the plane of twice the maintenance requirement, were decidedly lower than the above, being for alfalfa hay, 634 and 605 Calories, for corn meal, 2,494 and 2,477 Calories, and for the mixed ration, 1,557 and 1,531 Calories. These net-energy values of feeds for body increase apply only to that portion of the total fed which is in excess of the maintenance requirement.

The corresponding net-energy values at the plane of half more than maintenance were in either close or exact agreement with those at the higher plane of feeding for body increase.

The proportion of the gross energy eliminated as methane varied from 6.42 to 9.83 per cent, the corresponding proportions of heat of fermentation (computed according to Krogh, but with a question as to the method) being 1.50 and 2.30 per cent, respectively. The sum, that is, the proportion of the gross energy, lost as methane and heat of fermentation together were 7.92 and 12.13 per cent, respectively. The lowest value of each of the above categories was found in the periods in which hay alone was fed, and the highest values in the periods in which the feed was half of the maintenance requirement of the mixed ration of hay and grain.

From this study it appears that comparable determinations of the specific dynamic effects of feeds, foods, or nutrients, can be determined only at the same plane of nutrition.

An important unsolved problem in the determination of net-energy value of feeds is the interrelated or reciprocal effect of the components of a ration on their respective energy costs of utilization, especially as affected by the plane of nutrition, since it is not known that the heat-increment value of a ration is, at all planes of nutrition, the sum of the heat-increment values of its components.

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They classified the organism that they obtained as *Bacterium tumefaciens*, although it differed in cultural reactions and in reactions when inoculated on various hosts from the organism isolated from peach, daisy, and other hosts. They regarded the various forms as belonging to one species and described the organism isolated from the daisy as the type of the species. Attention is directed to the fact that the organism obtained from the apple by Smith et al. (*11*) failed to cause a reaction on the tomato inoculated with it. However, this organism infected the Paris daisy (*Chrysanthemum frutescens*). The reactions were not so pronounced as those obtained on this host by inoculations with the organism isolated from the peach.

That Smith and his coworkers recognized the fact that these organisms, obtained from different hosts and regarded by them as *Bacterium tumefaciens*, failed to cause infection when cross inoculated is evident, for under the caption "Quick tests for differential purposes" (*11, p. 115*), they recommend "inoculations into young, rapidly growing daisy shoots or into growing sugar-beet roots." It should be noted that the tomato, an easily grown host, was not recommended. These authors also continually refer to strains of *Bact. tumefaciens*, designating the daisy strain, the hop strain, and so on.

Riker and Keitt (*9*) and Muncie (*4*) noted the presence, in malformations on apple roots, of organisms that resemble *Bacterium tumefaciens*, but apparently all of their tests to determine the identity of organisms isolated from what they term "wound overgrowths" or malformations of any type were made on tomato, tobacco, or geranium, and not on Paris daisy, sugar beet, or Bryophyllum.

In the experiments reported here the daisy, as well as the tomato and other plants, has been used as a host to determine the identity of organisms isolated from malformations on apple-root grafts. For isolation purposes typical woolly-knot crown galls on the apple were selected. (Fig. 1, A, B, C, D.) This type of gall or malformation has almost invariably yielded an organism that appears to be identical with that described by Smith et al. (*11*) as the apple strain of *Bacterium tumefaciens* in its ability to cause reactions when inoculated into certain hosts. This so-called apple strain has been isolated from 38 of 52 galls similar to those illustrated in Figure 1. From the successful isolations, inoculations have been made into the daisy in every case and positive reactions have been obtained consistently. The types of reactions obtained on daisy and the controls are shown in Figure 2, A, B, C, and D. They generally consist of small intumescences ranging from one-eighth to one-quarter inch in diameter. Inoculations were made at intervals along the stem by means of single needle punctures from beef-agar cultures and the control punctures were generally made on the opposite side of the same shoot. In a number of cases the organism has been recovered from these galls and reinoculated into the daisy, resulting in infections.

A limited number of inoculations by means of single needle punctures from beef-agar slants have been made on small apple seedlings growing in the greenhouse. The seedlings were grown from open-pollinated fruit of the Chenango variety. Infections have been secured consistently with the apple organism, but none with the peach organism. Smith et al. (*11*), however, reported infection with the peach strain on apple. The type of infection secured on the apple in

STUDIES ON THE ETIOLOGY OF APPLE CROWN GALL[1]

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INTRODUCTION

The early history of crown gall on various hosts has been reviewed in detail by Hedgcock (*2*)[2] and by Smith, Brown, and Townsend (*11*). The stimulus imparted from their findings and the importance of this disease in California led C. O. Smith (*10*) to inoculate a number of hosts with the crown-gall organism (*Bacterium tumefaciens* Smith and Townsend) and thus to add further evidence of the cause and infectiousness of crown gall.

For almost a decade after Smith's publication (*11*), the hypothesis that practically all the various malformations that occur on root-grafted apple trees in the nursery were the result of infection by the crown-gall organism was generally accepted by plant pathologists. There were, it is true, a number of so-called doubtful cases, the etiology of which was not clear, but Riker and Keitt (*8*) in 1925 were the first to question seriously the current beliefs concerning the etiology of certain types of overgrowths found at graft unions and previously considered typical crown-gall formations. The experiments of Smith and his associates (*11*) on malformations of root-grafted apple trees were necessarily quite limited, and, as noted by Riker and Keitt (*9*) and later by Melhus (*3*), were not extensive enough to permit the drawing of definite conclusions.

Hedgcock (*2*) made an excellent classification, based on external characters, of the types of malformations on root-grafted apple trees. The type designated by him as woolly knot is the one studied chiefly by the writer. The woolly-knot type of malformation, which is the one most commonly found in the root-grafted apple nursery, is recognized by nurserymen and pathologists as numerically the most important. As shown by their illustrations, Riker and Keitt (*9*) were referring to this type when in 1925 they first reported their inability to obtain the crown-gall organism from a number of nursery trees rejected as crown-gall trees and when, somewhat later, they reported in detail their experiments and reiterated their previous findings, namely, that many types of malformation ordinarily diagnosed as crown gall were not due to infection by *Bacterium tumefaciens*, but were, in fact, wound overgrowths. Muncie (*4*) arrived at the same conclusion as the result of experiments performed along similar lines.

ISOLATIONS, CULTURES, AND INOCULATIONS

Smith and his associates (*11*) reported the isolation of an organism from what they termed the "hard gall" of apple, but it is not known definitely whether this was the woolly-knot or the hard-gall type.

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[1] Received for publication June 20, 1928; issued October, 1928. Credit is gratefully extended to R. B. Piper for most of the cultural and inoculation work in these experiments.

[2] Reference is made by number (italic) to "Literature cited," p. 313.

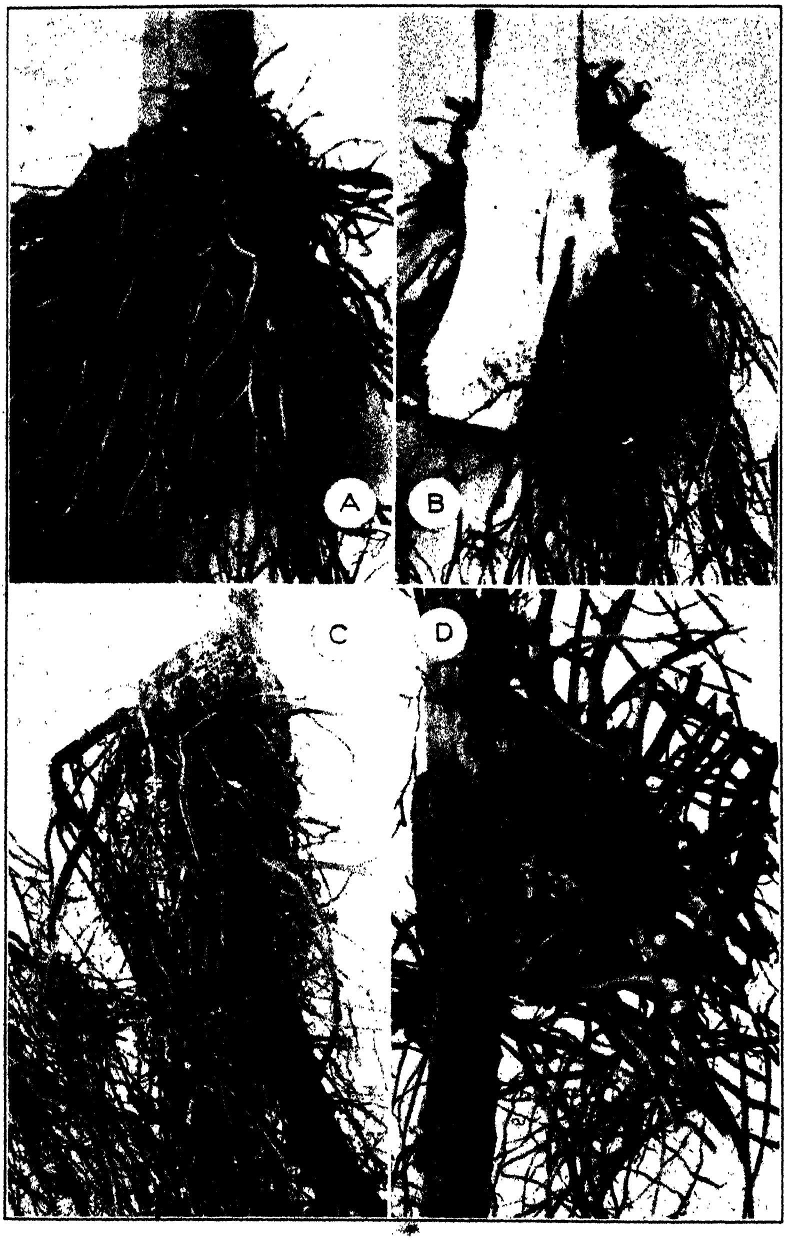

FIG. 1.—A, woolly-knot type of crown gall on 1-year-old Jonathan apple; B, longitudinal section of gall shown in A; C, woolly-knot type of crown gall on 1-year-old Stayman Winesap apple; D, woolly-knot type of crown gall on 1-year-old Wolf River apple. All natural size

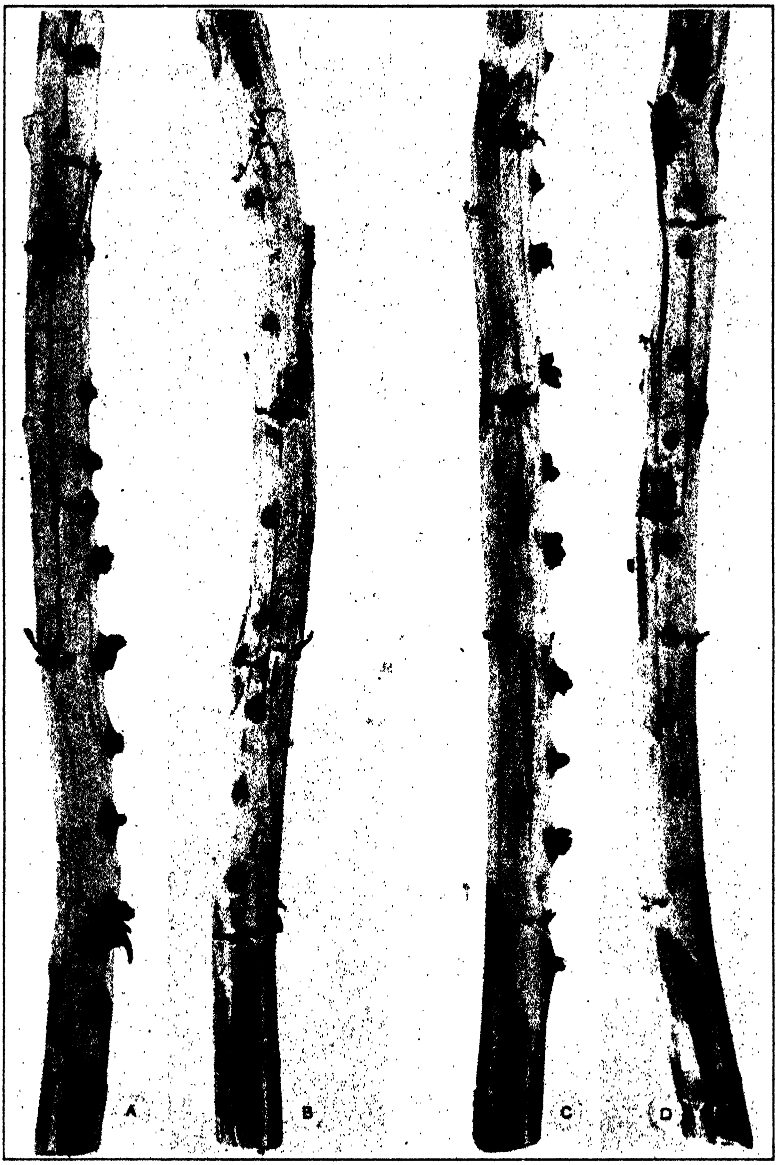

FIG. 2.—A, reactions obtained on daisy with the apple-strain organism by means of single needle punctures; time, 60 days. B, control punctures made on the opposite side of stem in A; time, 60 days. C, reactions obtained on daisy with the apple organism, isolated from gall shown in Fig. 1, D, by means of single needle punctures; time, 60 days. D, control punctures made on the opposite side of stem in C; time, 60 days. All natural size

the writer's experiments consists of a protruding mass of root primordia, morphologically identical with what is considered typical aerial gall. (Fig. 3.)

The organism has been recovered from the specimen illustrated in Figure 3 and reinoculated into daisy, resulting in infections. Invariably the control punctures have healed over. Brown (*1*) reported negative results from attempts at isolations from aerial galls from bearing trees. The galls from which successful reisolations were made in the writer's experiment were very young, that is, 45 days from date of inoculation, and were apparently in better condition to yield a pathogene. The type of gall produced by Smith et al. (*11*), and illustrated in their Plate 12, 2, and by Brown (*1*) in her Plate 1, B, is in marked contrast with the aerial type of gall produced in the present experiments. From the report of Taubenhaus's experiments[3] it is possible that he may have isolated *Bacterium tumefaciens* from aerial galls on apple, but, unfortunately, he does not report having checked his isolations by means of inoculations.

A limited number of sugar beets have been inoculated with the apple strain and infections have been secured in approximately 50 per cent of the trials. Young sugar-beet seedlings were grown with 1 inch of the crown exposed above the soil. The exposed portion of the root immediately below the crown was washed with mercuric chloride solution, then rinsed with sterile water, and the organism introduced by means of one needle puncture at five different places. The control needle punctures were made in similar positions on the opposite side of the root. Figure 4, A, shows the small, fleshy rootlike malformations produced on sugar beet by means of inoculations with the organism isolated from the apple gall illustrated in Figure 1, A. The control punctures are shown in Figure 4, B. In no cases have the controls shown the fleshy root formations illustrated here, although this type of root formation does appear infrequently on uninoculated roots, possibly through natural causes or natural infection. The reaction obtained is quite distinct from that resulting from inoculations with strains of crown gall obtained from hosts other than the apple. For comparison, attention is directed to Figure 4, C, which illustrates inoculation with a strain of *Bacterium tumefaciens* obtained from a peach gall. The apple-strain organism has been reisolated from the malformations produced on the sugar beet.

Inoculations made on the air plant *Bryophyllum calycinum* (Salisb.) with the apple organism have invariably resulted in malformations consisting of smooth, raised areas, approximately one-quarter inch in diameter. (Fig. 5, B.) The control punctures are shown in Figure 5, C. The reactions obtained on Bryophyllum by inoculations with the apple organism are more pronounced than those obtained on the daisy by inoculations with the same organism. They are not so pronounced as those obtained by inoculations with the peach organism. (Fig. 5, A.) In all instances, however, the inoculations with the apple-strain organism have been positive—that is, every needle puncture with the organism has resulted in infection—whereas none of the control punctures, made always on the opposite side of

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[3] Adams, J. F. Diseases of fruit and nut crops in the United States in 1922. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Sup. 28: 308, 1923. [Mimeographed.]

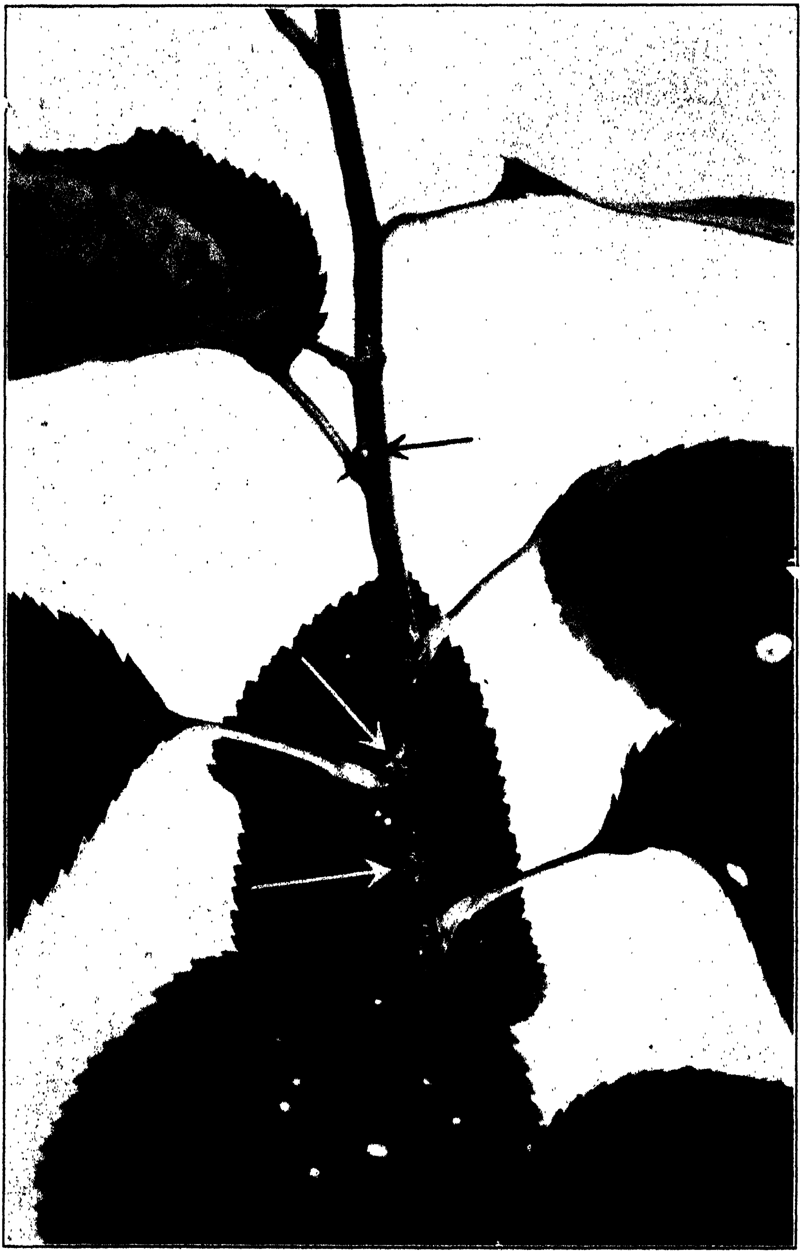

FIG. 3.—Reactions obtained on an apple seedling, grown from a seed of an open-pollinated Chenango fruit, by means of single needle punctures with the apple organism isolated from the gall shown in Fig. 1, C. These reactions are morphologically identical with the so-called aerial galls. Time, 45 days. Natural size

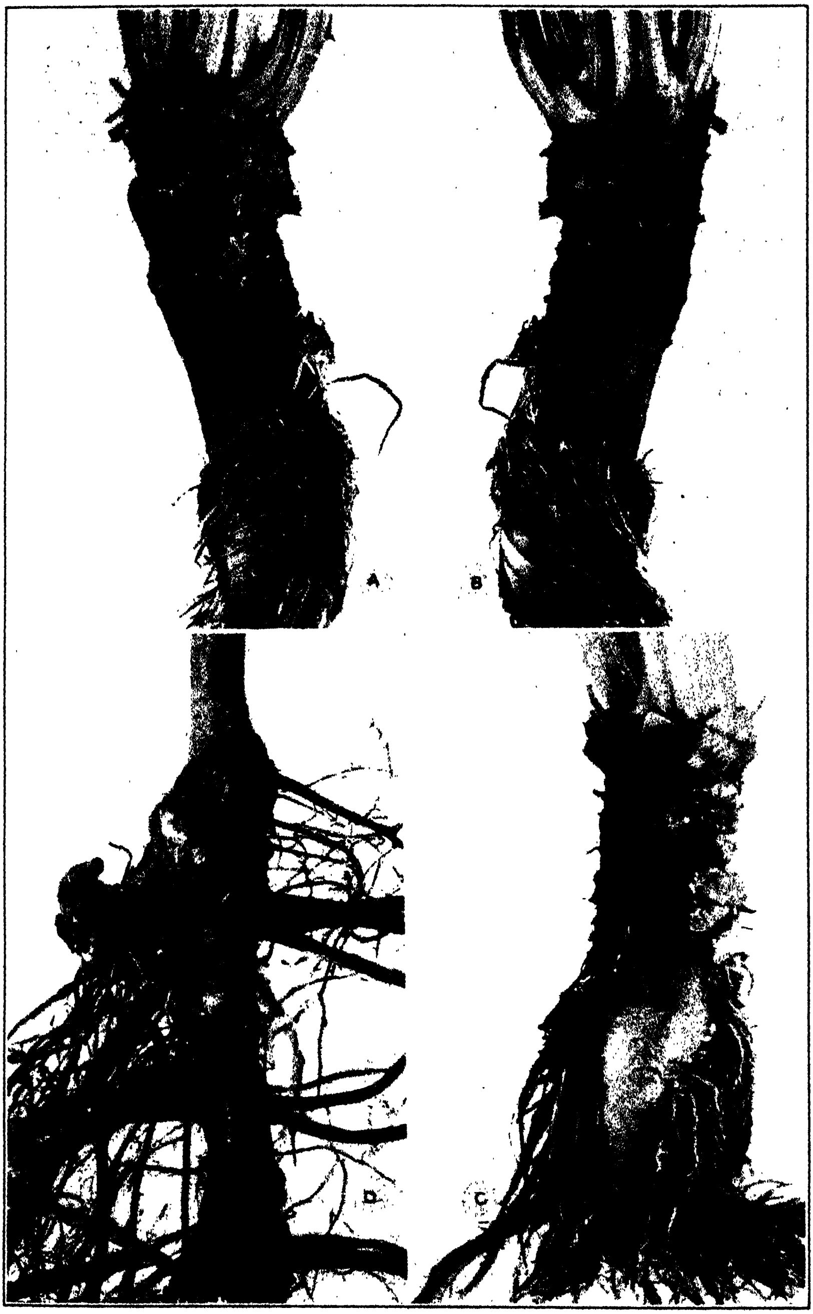

FIG. 4.—A, reactions obtained on beet seedling with the apple organism by means of single needle punctures; time, 60 days. B, control punctures on beet seedling on the opposite side of specimen shown in A; time, 60 days. C, reactions obtained on beet seedling with the peach strain of *Bacterium tumefaciens;* time, 60 days. D, malformation on a 1-year-old Duchess graft, from which the apple organism was obtained. All natural size

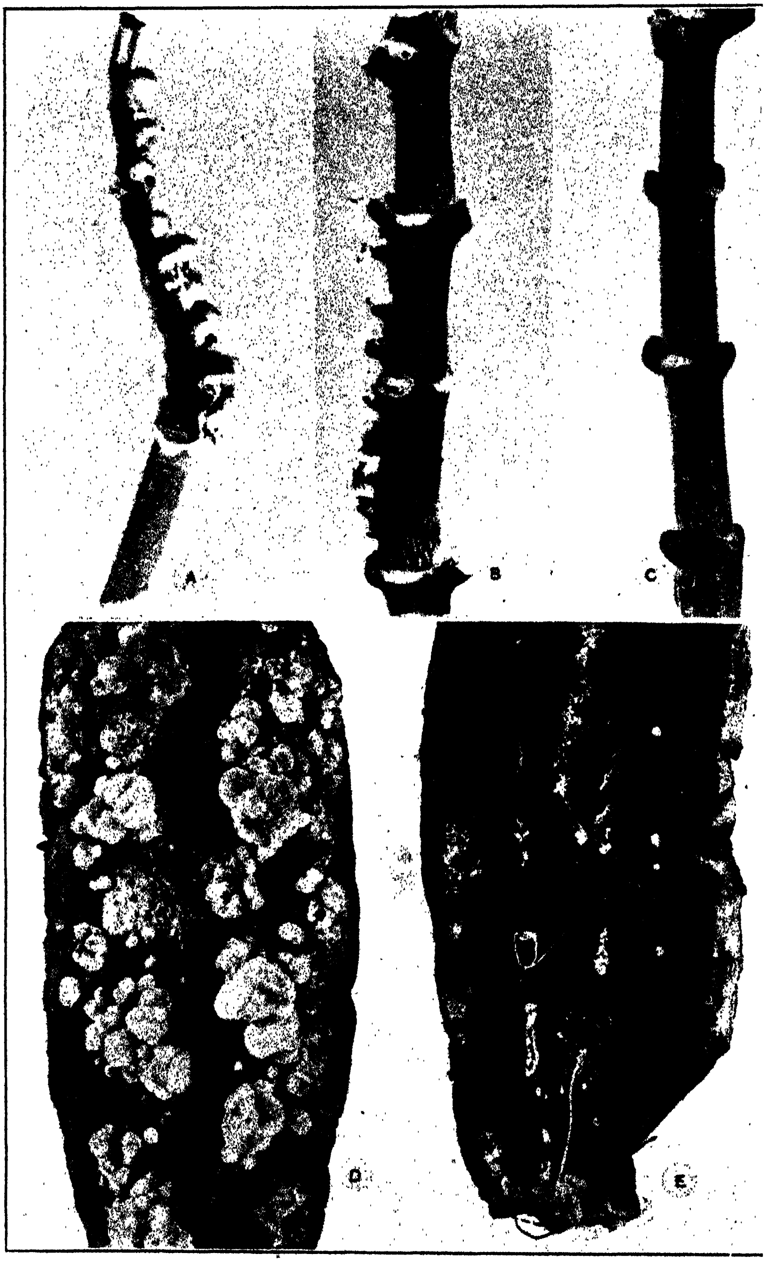

FIG. 5.—A, reactions obtained on Bryophyllum by means of single needle punctures with the peach organism; time, 30 days; × ¾. B, reactions obtained on Bryophyllum by means of single needle punctures with the apple organism isolated from the specimen shown in Fig. 1, C; time, 30 days; × ¾. C, control punctures, made on the opposite side of the stem shown in B; time, 30 days; × ¾. D, intumescences obtained on cut surfaces of raw carrot by inoculation with the peach strain of *Bacterium tumefaciens;* time, 35 days; × 1½. E, intumescences obtained on cut surface of raw carrot by inoculation with the apple organism; the areas appearing "frosty" were caused by normal cell proliferation; time, 35 days; × 1½

the stem, has caused the slightest reaction. The organism has been recovered at will from the malformations produced on Bryophyllum by artificial inoculations, and plates poured from these areas have been practically pure.

Smith et al. (*11*) illustrated the intumescences obtained by inoculating cut raw surfaces of turnip with the daisy organism. Through correspondence the writer was informed that C. Stapp, of Germany, inoculates the cut surfaces of raw carrots in Petri dishes as a convenient method of testing the crown-gall organism. The writer, following this method, has made inoculations on carrots and has secured large intumescences on this tissue by inoculations with the peach strain (fig. 5, D), but has secured only slight intumescences approximately one-eighth inch in diameter by inoculations with the apple strain (fig. 5, E).

Inoculations into tomato, tobacco, geranium, and coleus with the apple-strain organism have been considered negative, although inoculation with the strain of *Bacterium tumefaciens* isolated from the peach always results in positive reactions on these hosts. The number of inoculations with the apple organism on geranium and coleus, however, has been too limited to permit the drawing of definite conclusions. Although inoculations on the tomato with the apple organism are considered negative, this statement should be qualified, since slightly water-soaked areas, absent from the control punctures (fig. 6, C), appear about the points of inoculation (fig. 6, B), followed by a slight disturbance of the tissues, as is shown in Figure 6, E. This plant was photographed approximately five months after the date of inoculation and the organism was recovered in practically pure culture.

Inoculations on the tomato and on the daisy with the peach organism are illustrated in Figure 6, A and D, respectively. A comparison between Figure 2, A and C, and Figure 6, D, is invited.

Since the results of inoculations depend so greatly upon the condition of the host, the inoculations made in these experiments are not tabulated. The general practice has been to make at least 10 needle punctures on the daisy stem with organisms isolated from the apple. Control punctures were made in the same manner. If only one of the inoculations resulted in infection the organism was considered the apple strain. Usually about 5 of the 10 points of inoculation resulted in infections. Whenever there was any doubt about an isolation it was subjected to repeated tests so that the result of hundreds of inoculations leaves no doubt about the ability of the apple organism isolated from the 38 galls of the woolly-knot type to perform as indicated here.

STRAINS OF THE CROWN-GALL ORGANISM

It has been deemed advisable to follow the suggestion of Smith et al. (*11*) and to call the organism obtained so consistently from the woolly-knot type of crown gall on apples the apple strain of *Bacterium tumefaciens*. These workers recognized and discussed in detail strains of the crown-gall organism and, although they concluded that "the ease with which . . . cross-inoculations take place points rather to one collective species," they also stated that "the differences [in the

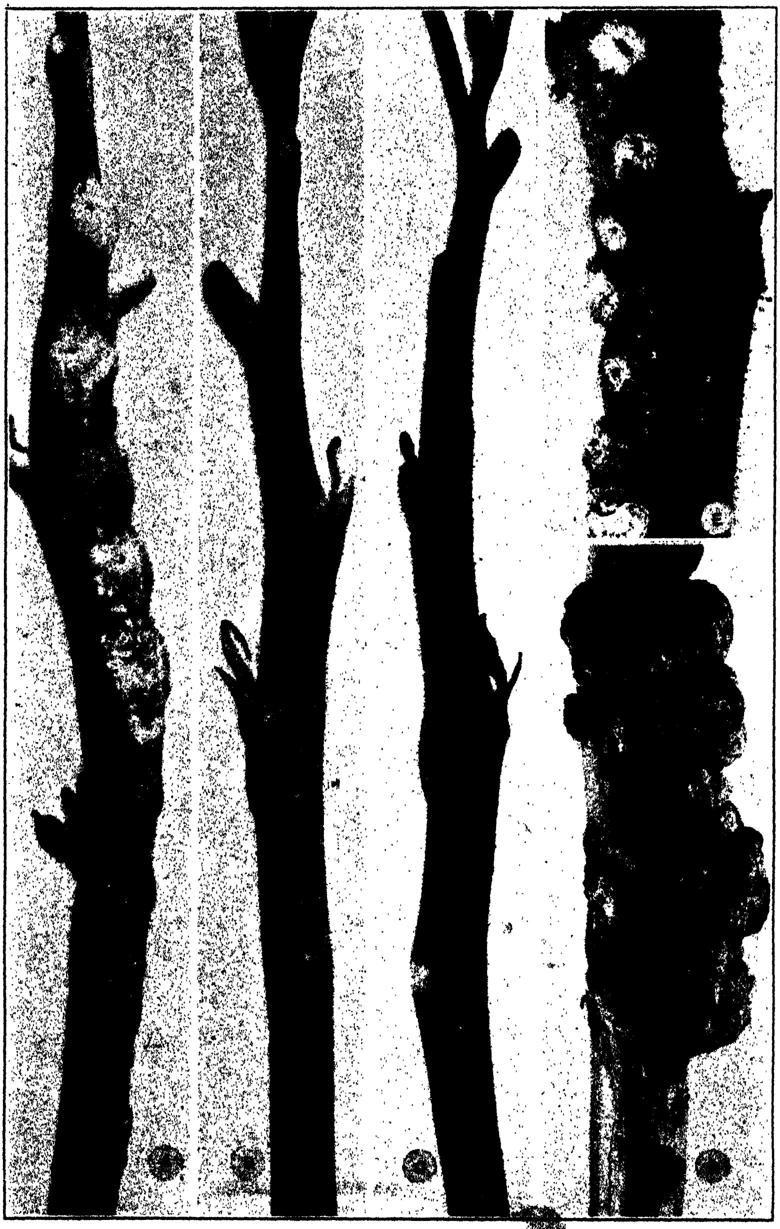

FIG. 6.—A, reaction obtained on tomato by means of single needle punctures with the peach strain of *Bacterium tumefaciens;* time, 30 days; × 1¾. B, slightly water-soaked areas (barely discernible in this illustration) at points of inoculation of the tomato with the apple organism; time, 30 days; × 1¾. C, control punctures on tomato; time, 30 days; × 1¾. D, typical malformations obtained on daisy by inoculations with the peach strain of *B. tumefaciens;* time, 90 days; × 1¾. E, slight disturbances on tomato at points of inoculation with the apple organism; time, 135 days; × 2

strains] . . . seem to be real differences, . . . [and] we do not know . . . what weight to give them as differential characters."

The conception that these several strains belong to one widely variable species is in conformity with the general conception of bacterial organisms. For example, the root-nodule organism (*Bacillus radicicola*) is generally accepted as consisting of several different strains, either partly or not at all cross infectious.

While it is probable that the organism repeatedly isolated from the apple in these experiments is identical with the one isolated from it by Smith et al. (*11*), this belief may be unwarranted, as they (*11, p. 100*) evidently believed that the culture they used for the determination of cultural characters had become contaminated. This is rather confusing since the organism used in these experiments agrees in cultural reactions with their apple organism. The fact that the cross inoculability of the two organisms agrees strengthens the belief that the two are identical, but the doubt as to their identity can not be dispelled in the absence of a comparison of the two organisms.

Patel (*5, 6*) has reported on the isolation of 15 strains of *Bacterium tumefaciens*. The organism isolated by Riker and Keitt (*9*) and by Muncie (*4*) from a type of gall occurring infrequently in the nursery, apparently is identical with the daisy organism of Smith et al. (*11*), if the ability to infect not only the daisy but also the tomato, the tobacco, and the geranium is accepted as a criterion.

That the reaction on culture media apparently is not always a reliable test for the crown-gall organism was noted by Riker (*7*). The organism isolated by him from the raspberry turned litmus milk pink, whereas the daisy organism, as had been noted by Smith and others, never did so. Riker (*7*), referring to certain morphological differences and cultural reactions, also calls attention to the fact that Smith had noted variations "as great as these between different strains of crowngall bacteria."

For the present, despite any morphological or physiological differences, it is deemed advisable to consider the organism used in these experiments as the apple strain of *Bacterium tumefaciens*.

DISCUSSION

Knowledge of the etiology of certain types of malformations found in root-grafted apple trees can not be fully rounded out until apple grafts can be grown in bacteriologically sterile soil.

The practical difficulties encountered in growing apple grafts on an extensive scale under aseptic conditions are well recognized. Riker and Keitt (*9*) and Muncie (*4*) used extreme care in an attempt to obtain aseptic conditions under which to grow apple grafts. Riker and Keitt (*9*), in a cultural examination of some of the trees resulting from these grafts, reported that "all were negative for the crown gall organism," but stated that some of the grafts examined were not sterile. Muncie (*4*), in an experiment conducted similarly, reported the absence of *Bacterium tumefaciens* but at the same time noted the presence of bacteria in the galls examined, and called attention to the fact that the plates "were flooded . . . and [then the suspension] inoculated into tomato [plants]" with negative results. Here again the tomato and not the daisy was used to test the organisms that were isolated. Thus it is evident that Riker and Keitt (*9*) and

Muncie (*4*) were unable to obtain sterile conditions for growing apple grafts and that their isolations from so-called wound overgrowths were tested on hosts that are not susceptible to the organism isolated by the writer. But regardless of these facts it should be noted that Riker and Keitt's Plate XLV, E, (*9*) and Muncie's Plate III, B, (*4*) do not represent the types of malformations discussed in this paper. In other words, they are not the woolly-knot type of crown gall. They are considered not to be the types of malformations commonly encountered in the nursery and greatly resemble the specimen illustrated in Figure 4, D, which is a Duchess apple tree grown from a graft with a very poor union. While the apple strain of the crown-gall organism actually was isolated from this specimen, it is still possible that callus formation of nonpathogenic origin may have played the more important rôle in causing this malformation. Patel (*6*) and others have isolated the crown-gall organism from the soil and it is conceivable that the organism might be recovered quite readily from apparently healthy graft unions, just as the pear-blight organism (*Bacillus amylovorus*) often may be found streaming over healthy bark.

Just how important a rôle this apple strain of *Bacterium tumefaciens*, if in fact it is a strain of that organism, plays in the formation of the woolly-knot type of malformation is still undetermined. Evidence is produced showing that it is quite constantly associated with these malformations; that it is pathogenic to a limited degree on daisy and apple shoots, as well as on sugar beet, and causes malformations on them, and that it produces even more pronounced malformations on Bryophyllum. It will produce, however, only a barely perceptible reaction on tomato and tobacco. It has also failed, in limited tests to cause a reaction on geranium. These hosts were used by Riker and Keitt (*9*) and by Muncie (*4*) for testing organisms that they isolated from malformations designated by them as wound overgrowths. Most of these overgrowths apparently are identical with the type of crown gall known as woolly knot and used in these experiments.

SUMMARY

Smith, Brown, and Townsend isolated a bacterial organism from malformations on root-grafted apple trees. This organism, which was designated as the apple strain of *Bacterium tumefaciens* Smith and Townsend, caused local reactions when inoculated into the Paris daisy, but no reaction when inoculated into the tomato.

Isolations have been made from types of malformations on grafted apple trees, known as woolly-knot crown gall. Isolations from these types of malformations have consistently yielded an organism that appears to be identical with the organism which Smith et al. designated the apple strain of *Bacterium tumefaciens*.

When Paris daisy, apple shoots, sugar beet, and Bryophyllum are inoculated with the apple organism, definite and pronounced galls or malformations are produced, whereas when tomato and tobacco are inoculated with this organism no definite galls are produced, although extremely slight distrubances of the tissues occur on these hosts. These results demonstrate the necessity of using the proper host, in receptive condition, in testing the infectiousness of organisms isolated from malformations on the roots of apple trees.

When apple shoots are inoculated with the apple organism malformations morphologically identical with the so-called aerial crown gall are produced. The organism has been reisolated from these artificially produced aerial galls.

The degree of pathogenicity that this apple strain of *Bacterium tumefaciens* exhibits on apple shoots and on host other than the apple does not necessarily prove its rôle as a pathogene in connection with malformations on the roots of grafted apple trees; nor does its constant association with these malformations furnish conclusive proof that it is the causal agent. These facts, however, should be included in any consideration concerning the nature or cause of these malformations.

While emphasis is placed on the desirability of growing apple grafts in bacteriologically sterile soil before passing full judgment on the question of the etiology of these malformations, the facts given here are considered to support the hypothesis that the apple strain of *Bacterium tumefaciens* can cause certain types of malformations or galls which occur on root-grafted apple trees.

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THE INFLUENCE OF CHILLING, ABOVE THE FREEZING POINT, ON CERTAIN CROP PLANTS[1]

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INTRODUCTION

It has been held that short periods of cold weather above the freezing point cause injury to crop plants of tropical or subtropical origin. It is commonly known that growth is retarded by such temperatures but it does not seem to be generally conceded that definite lesions may result. The authors, therefore, thought it worth while to investigate the effect of temperature near, but above, the freezing point on certain crop plants and, if injury were found to occur, to study the relative susceptibility of different crops and varieties and the circumstances under which injury takes place.

REVIEW OF LITERATURE

Very little recent literature has been contributed to this subject. Molisch (*12*),[2] 1896, critically reviewed the earlier literature, making special reference to the work of Bierkander (*2*), Goeppert (*7*), Hardy (*8*), Sachs (*19*), and Kunisch (*10*), and conducted rather extensive experiments of his own. Some 58 plants from subtropical regions were examined and found to be injured by temperatures ranging from 1° to 7° C. Some showed characteristic black spots on their leaves; in others the leaves rolled and ultimately dropped off, some plants died and some survived without injury.

Sachs attributed the injury to the inability of the roots to absorb and convey sufficient water from the cold soil to the leaves to make up for the transpiration deficit and hence the plants died of drought. But Molisch disproved this for *Episia*, *Sanchezia*, and some 10 other plants, by showing that even when they were kept in air having an average humidity of 98 per cent, thus reducing transpiration to a minimum, the characteristic injury nevertheless occurred.

Faris (*5*), working on sugar cane in Cuba, reported the appearance of "mancha blancas" or white bands 2 to 4 inches wide across the leaves in which the tissues seemed to have lost the power to form chlorophyll. A study of the weather conditions showed that the bands occurred when low temperatures followed periods of rainfall. He thought that rain stimulated growth and the cold water standing in the curl of the leaves chilled the newly exposed tender tissues and thus caused chlorosis. Cold weather when not preceded by rainfall caused only slight chlorotic bands. Marked varietal differences were found and some 20 varieties were arranged on a scale of 1 to 10, according to their resistance. In Habana Province, where this study was made, no freezing temperatures have ever been recorded. He concludes that a temperature from 5° to 10° C. for two to three nights is sufficient to cause white bands on susceptible plants. According to Faris, cold chlorosis has also been reported from Australia, Hawaii, and Louisiana.

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[1] Received for publication June 1, 1928; issued November, 1928. Contribution No. 175, Department of Agronomy, Kansas Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 337.

Faris was able to produce similar bands artificially by tying a paper funnel around the plant shoot and filling it with ice for three consecutive nights. The white bands developed six days later.

Collins (*4*) found that albinism in certain varieties of barley could be induced by growing the plants at temperatures below 45° F. (7.3° C.). When the plants were grown at temperatures above 65° chlorophyll developed normally.

Collings (*3*) states that cool nights, but without frost, injure, dwarf, and delay growth of young cotton plants.

Marcarelli (*11*) observed that the yellowing of the young plants of late rice, irrigated with cold water in the upper Vercelli district, Piedmont, Italy, was caused by frequent early morning low temperatures accompanied by mist.

Pantanelli (*15*) studied the relation between various salts and the injury to plants exposed to temperatures near the freezing point. Wheat, beets, and sunflowers were exposed to freezing temperatures and maize and tomatoes to temperatures "a little above 0° C." Different lots in each case were supplied with sodium, potassium, ammonium, and magnesium salts. The same relations were observed whether death resulted from freezing or from cooling alone. The author concludes that "resistance to cold has, therefore, no connection with the concentration of the cell sap, nor with its content in acids or salts, but with the amount of sugar retained by the cell during cooling."

EXPERIMENTAL ~~RESULTS~~ [illegible]

The investigations reported herein were carried out during the summer of 1927 in the greenhouses of the agricultural experiment station, Manhattan, Kans. Good ventilation was afforded and excessive temperatures were prevented by covering the glass with a coating of whitewash (calcium hydroxide) which, however, excluded very little light. The plants not subjected to chilling or other abnormal conditions made normal growth and seed produced. Even soy beans, which often grow thin stemmed and decumbent in greenhouses, were in no way different from field-grown plants.

The plants were grown in wooden flats or boxes 24 by 24 by 4 inches and in about 2,000 red clay 4-inch greenhouse pots. They stood for most of the time on the greenhouse floor. Their position was changed every third day to equalize conditions dependent upon location. Thick plantings were made and later thinned to one sturdy plant to each pot or each 12 square inches in the flats.

Soy beans and velvet beans were germinated in coarse quartz sand and the more vigorous seedlings transplanted to pots on account of the scarcity and low vitality of the seed. Twenty-five to one hundred pots were planted at intervals throughout the time these experiments were in progress, with the object of having plants 2 to 3 weeks old whenever needed.

The chilling was effected in a chamber specially constructed in the greenhouse for low-temperature investigations. (Fig. 1.) Its walls are constructed of sheet cork, protected on the outside by plaster and cement, the entire thickness of cork and plaster being 12 inches. The chamber was provided with three doors, each 42 by 24 inches, which fitted snugly into the openings they covered. These doors were closed during the night only. During the day they were replaced by double glass fitted into wooden frames, the glasses being separated by a space of 3 inches. The chamber was approximately 10 feet long, 4 feet wide, and 3 feet deep. The coils cooling the chamber were 6 inches away from the four walls and the floor, leaving a space of approximately 67 cubic feet between them.

Wooden boards were placed lengthwise on cross pieces 6 inches from the floor. On these boards the pots were placeo in juxtaposition and at least a pot diameter away from the coils. In no instance were the plants allowed to touch the coils. Plants from each set similarly treated were placed across the chamber so that each group had an equal number of plants in the middle and toward the sides. This precaution proved unnecessary since comparisons of three thermometers over a period of four days indicated that there was no appreciable difference in temperature for any length of time in different sections of the chamber.

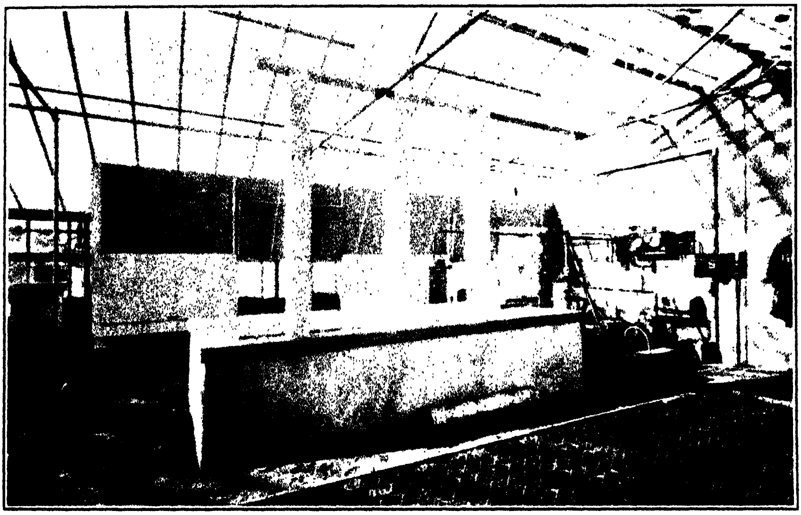

Fig. 1.—Cold chamber and refrigeration plant used in chilling crop plants

Cooling was effected by a carbon dioxide refrigeration plant thermostatically controlled. By this arrangement it was possible to maintain the desired temperatures within a range of about three centigrade degrees. The temperatures within the chamber and in the greenhouse were continuously recorded by two Tycos thermographs, which were checked twice daily with minimum and maximum thermometers suspended near them.

Watering of the plants before and after chilling was done daily but at no regular time, approximately 40 to 50 c. c. of water being applied to each plant. In all instances (except where the quantity of water used was the subject of investigation) water was applied immediately before the plants were placed in the chilling chamber. While the plants were being chilled they received no water. Those pots which were saturated prior to chilling were watered a sufficient time before chilling to allow all the free water to drain off.

The humidity of the air within the chamber ranged, as measured with a Tycos hygrodeik, between 95 and 98 per cent. Sufficient moisture condensed on the refrigeration pipes to warrant a triweekly scooping out of the water from the chamber floor. While the pots were in the chamber they remained so moist that watering was unnecessary even over a five-day period. It seems likely that very little if any transpiration took place.

The opening of the doors, replacing and lifting of the glass frames, together with fluctuating temperatures in the chambers, no doubt forced in and withdrew sufficient air to effect a regular exchange between the inside and the outside. Under such circumstances the air surrounding the plants was not very different from that in the greenhouse.

METHOD OF RATING INJURIES

The amount of injury, retardation of growth, and other abnormalities induced by chilling were recorded, in most instances, within 24 hours after the plants were removed from the chamber while the immediate effects were most clearly visible, and again after two or three weeks. All records of injury were made on a percentage scale. Each species reacted in its own particular way, and this fact had to be considered in estimating the amount of injury that had taken place. The first notes were based on the proportion of leaf and stem surface showing visible injury. Final notes were based on the estimated permanent injury to the plant. Thus a plant having a few dry tips or drooping petioles, depending on the total number of leaves of the plant, was rated as 5 to 10 per cent injury; whereas a total loss of all leaves might be rated at 95 per cent and of the stem and all leaves at 100 per cent. After a little practice there appeared to be no difficulty in assigning approximately the same figure to plants which had been similarly injured at different times. As a rule plants recorded soon after chilling as having suffered 25 per cent injury or less recovered and were capable of seed production. Those injured more than 25 per cent and less than 50 recovered occasionally. Plants recorded as 50 per cent or more injured seldom recovered.

SCOPE OF THE INVESTIGATION

Preliminary tests were first conducted to find what plants might be expected to suffer injury and what temperature and periods of exposure were necessary to induce such injury. More careful experiments were then undertaken to determine not only the temperature and duration necessary to produce injury, but also the relative susceptibility of different crops and varieties as well as the effect of various salt solutions on the degree of injury. The effect of varying amounts of water in the soil was also investigated. Summer crop plants, mostly of subtropical origin sensitive to the least degree of frost, were included in this study. Unless otherwise indicated they were grown from Kansas-produced seed. The following plants were used:

Cowpeas. *Vigna catjang sinensis:* Early Buff and Whippoorwill varieties from Georgia.

Velvet beans. *Stizolobium deeringianum:* Ordinary mottled variety from Georgia.

Peanuts. *Arachis hypogaea:* Valencia and Virginia Bunch varieties; source of seed unknown. Spanish from Georgia.

Soy beans. *Soya max:* Manchu and Virginia varieties.
Cotton. *Gossypium hirsutum:* Westex from Texas. Trice from North Carolina. Delfos from Mississippi. Oklahoma Triumph from Oklahoma.
Sunflower. *Helianthus annus:* White-seeded variety.
Tomato. *Lycospersicum esculentum validum.*
Potato. *Solanum tuberosum.*
Flax. *Linum usitatissimum.*
Watermelons. *Citrullus* sp.
Pumpkin. *Curcubita pepo.*
Tepary bean. *Phaseolus acutifolius* (A. Gray) *latifolius* (Freeman).
Buckwheat. *Fagopyrum esculentum.*
Maize. *Zea mays:* Midland Dent, Colby and F_1.
Kansas sunflower Hybrid No. 5413–1 × 5412–1.
Sorghums. *Holcus sorghum.* Blackhull kafir, Kansas Orange.
Rice. *Oryza sativa:* Early Prolific and Honduras varieties from California.
Sudan grass. *Andropogon sudanense.*
Teff grass. *Eragrostis abyssinica:* From the Union of South Africa.

EXPERIMENTAL RESULTS

The outstanding result of the investigation was the very evident injurious effects of chilling [13] on certain plants and the high degree of resistance shown by others. A point of considerable interest was the reaction of different species and varieties not only to difference in the degree of temperature, but also with respect to the nature of the injury. It seems desirable, therefore, to describe briefly the nature of the injury in each case.

NATURE OF THE INJURIES

The most obvious effect of chilling on susceptible plants was the drying and falling off of the leaves or portions of leaves in a way very similar to that which follows frost damage. The effect, however, in all except velvet beans, took place much more slowly, being apparent only from one to several days after chilling. In velvet beans, the effect was visible an hour or two after chilling when the leaves of young plants showed light purplish or purplish to black discoloration. This condition was followed by the drying of the leaves in the course of two to three days. When the exposure was brief there was no immediate effect, but in the course of five to seven days light brown areas appeared on the leaves. Plants 4 to 5 weeks old showed no abnormalities at first, but in a day or two a very pronounced chlorotic condition set in throughout the entire plant, from the top downward, accompanied by the dropping of the turgid leaves.

In most cases the effect on cowpeas was not immediately visible, except that when they came out of the chilling chamber the leaves were partly folded. A chlorotic condition, confined to the older leaves and more especially to leaf sections and similar to that in the velvet bean plants, usually developed within 36 hours. The leaves dropped while turgid and for the greater part still green. Slight injuries were evident in the form of disrupted or blistered areas confined to the edges of the younger leaves. (Pl. 1.) These areas eventually dried, turned brown, and disintegrated, leaving a leaf with a jagged outline. Cowpea plants were seldom killed outright, although the leaves were easily injured and the growth of the roots was considerably reduced. (Fig. 2.) New growth was generally produced from uninjured stem buds.

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[13] Chilling in this study refers in all cases to temperatures above zero centigrade. In no case was the temperature allowed to go below zero.

The after effects of chilling manifested by cotton and soy beans were very similar, although as pointed out later, there was the most marked difference in the degree of injury. The most striking effect was the presence of spots or fringes of light green which later became white and coriaceous. To the casual observer such leaves would seem to have been injured by frost except that the entire leaf was seldom affected. In severely injured leaves and occasionally in others a narrow brick-red band separated the living and dead portions of the leaves. (Pl. 1.)

Peanuts showed no obvious effects immediately after chilling. After about three days the tops wilted more and more during the afternoons, recovering somewhat at night. Wilting and recovery alternated until the plants died or recovered completely. Later examination showed that the root system had been seriously injured.

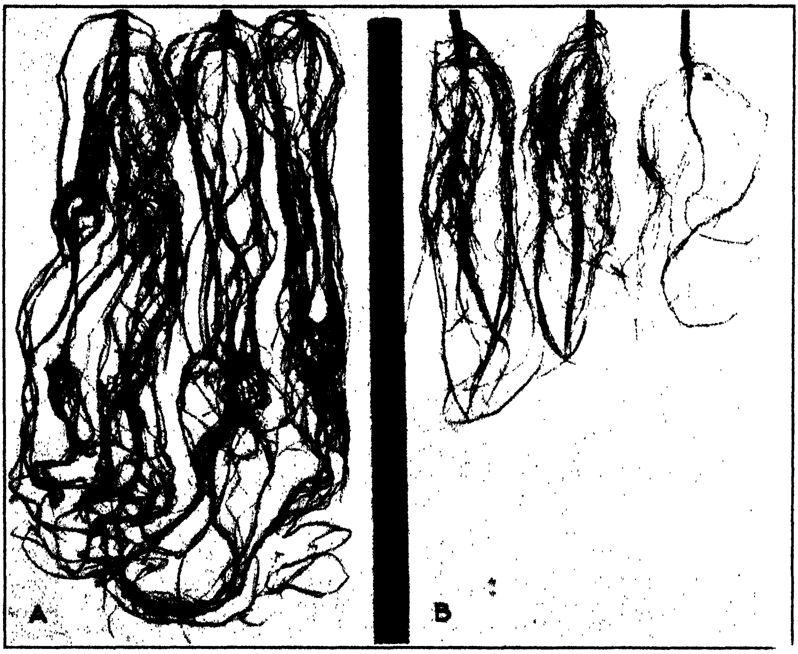
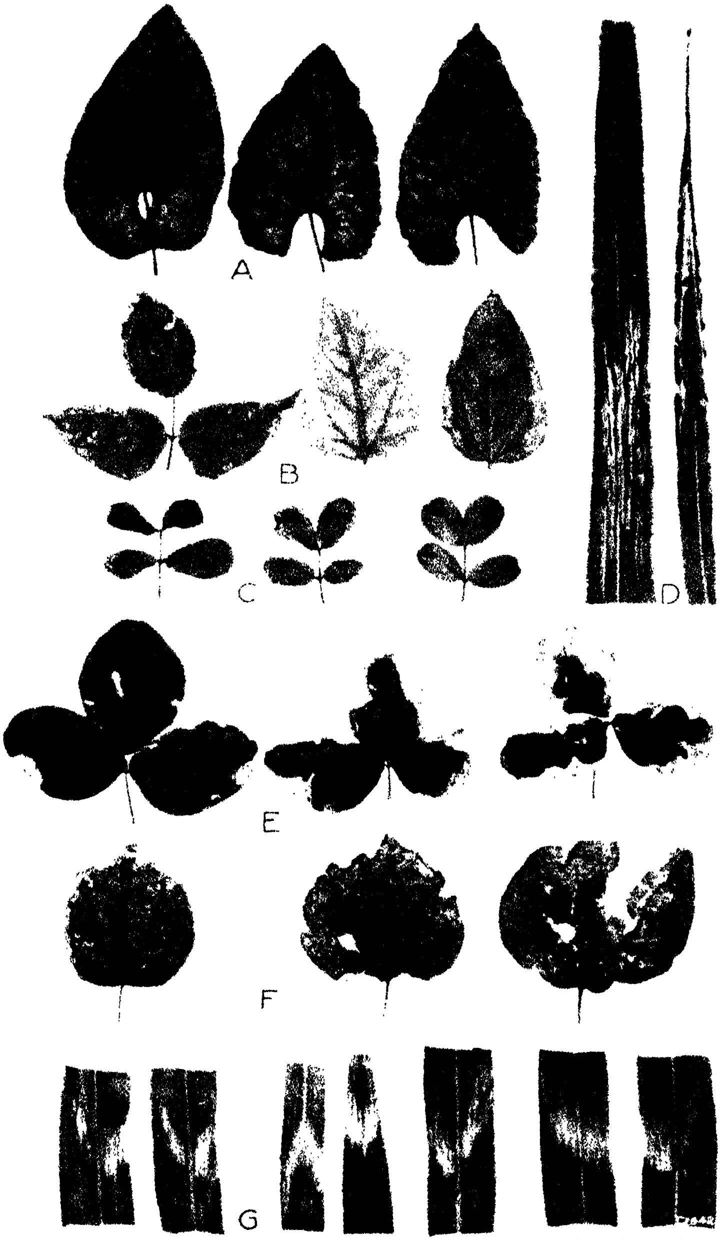


FIG. 2.—The effect of chilling on the roots of 3-week-old Whipporwill cowpea plants. A, not chilled; B, chilled for 36 hours at 2° to 4° C. Plants 6 weeks old when photographed

(Fig. 3.) In the case of young Virginia Bunch peanut plants a few leaflets developed narrow, dry brittle borders. More often individual leaflets became much dwarfed in comparison with the normal ones next to them. (Pl. 1, bottom.) In general the tops were uninjured. Injuries to the root systems, however, as will be shown later, were sufficient greatly to retard growth and in some cases to cause the death of the plants.

In watermelons and pumpkins the margins of the leaves turned light green and ultimately brown. When severely chilled the plants were unable to remain upright, the leaves then turning slightly yellow and folding inward. Chilling very clearly decreased the rate of growth but otherwise there was no marked effect either on the roots or leaves.



TYPES OF INJURY SUSTAINED BY VARIOUS PLANTS WHEN CHILLED

A, velvet bean basal leaves, a leaf from a slightly injured plant and two leaves from severely injured plants; B, Early Buff cowpeas, terminal leaf from a slightly injured plant and two leaves from severely injured plants; C, peanut leaves, showing characteristic dwarfing of leaflets and fringing that occurs after chilling; D, irregular Faris band on Kansas Orange sorghum leaf and fringed tip of the same; E, leaves from Virginia soy-bean plants which were chilled 48, 72, and 96 hours, respectively; F, Delfos cotton leaves which were chilled 48, 72, and 96 hours, respectively; G, Faris or chill bands on maize leaves from plants that have been chilled 60 hours, at 2° to 4° C.

Buckwheat and Tepary beans were severely wilted during and immediately after chilling, appearing as though they had been frosted. They recovered later to the extent that after a week or two scarcely any injury was perceptible.

Potatoes, tomatoes, and flax showed no appreciable injury or retardation in growth in any part during or after chilling, even when chilled as long as 120 hours. In a few tomato plants some half-developed leaf tips lost their turgidity and became pale green. Recovery in every case was very rapid.

Chilling had no marked immediate effect on maize, sorghum, Sudan grass, or Teff grass. From 5 to 10 days after chilling there developed on the leaf blades light yellow transverse bands, varying in size and intensity of color, similar to those described by Faris (*5*) in sugar cane. Their breadth and number per plant were proportional to the duration of the chilling. With advancing age these areas became transparent, filmy, and rust colored toward the edges. (Pl. 1, bottom.) It was evident that these bands occurred on those parts of the blades which formed the curl of the plant at the time of chilling. In this region the most active growth takes place and, incidentally, here the youngest and most easily injured tissue is to be found. Plants severely injured allowed their leaves to roll or droop as a browning of the entire plant set in and when dead appeared not unlike plants that had succumbed to drought or to intense heat.

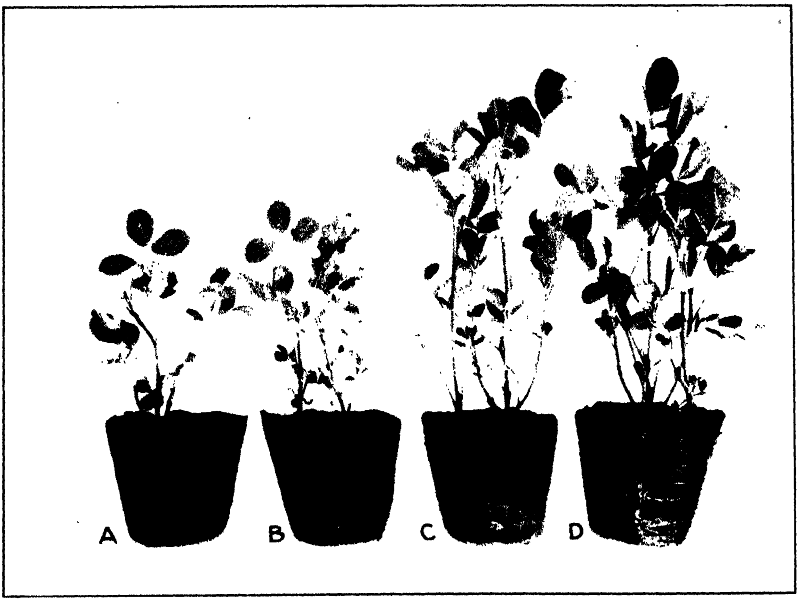


FIG. 3.—Retarded growth of Spanish peanuts due to root injury caused by chilling. A and B, chilled for 84 hours, C and D, not chilled

Microscopic examination of maize root sections from chilled and unchilled plants showed brown lesions on the roots of chilled plants only. Mycelia were found in apparently healthy root sections of chilled plants, while plants not chilled failed to reveal any abnormalities either internally or externally.

Unlike the foregoing, rice plants became yellow first in the leaf sheaths and then in the upper halves of the blades. When the chlorotic condition was confined to the sheaths, the plants recovered but when more extensive they perished. They did not develop Faris bands, nor did they show injuries on the roots. The yellowed sheaths invariably peeled away from the leaves they enveloped, becoming somewhat white, while their blades developed streaks of yellow and faint red. Cessation of normal functioning and discoloration took place alike throughout the entire plant, without any one part showing especially characteristic markings of its own.

RELATION OF PERIOD OF EXPOSURE TO INJURY

One of the first points to be investigated was the duration of exposure necessary to produce damage. For this study various plants were exposed to temperatures slightly above freezing for various periods of time ranging in general from 12 to 132 hours. Six to ten plants were used as a unit and the temperature in the chilling chamber was 0.5° to 5° C. in most cases. Exceptions are noted elsewhere. Tables 1 to 3 give averages of the estimated percentage injury sustained by the plants, as well as the least and greatest percentage injury in each set.

TABLE 1.—*The effect of chilling at 0.5° to 5.° C. on legumes*

Length of time chilled at 0.5° to 5° C. (hours)	Injury to plants: 24 hours after chilling: Average per plant	Injury to plants: 24 hours after chilling: Range	Injury to plants: 2 weeks after chilling: Average per plant	Injury to plants: 2 weeks after chilling: Range	Height of plants 4 weeks after chilling: Average	Height of plants 4 weeks after chilling: Range
VELVET BEANS (3 WEEKS OLD)						
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
12	0	0-00	4.4	0-10		
24	11.4	8-30	46.4	30-100		
36	42.4	10-50	80.0	60-100		
48	60.3	10-90	98.0	100-100		
EARLY BUFF COWPEAS (3 WEEKS OLD)						
12	0	0-0	3.0	3-3		11-13
24	3.0	0-9	10.0	10-10		13-14
36	26.5	15-30	20.0	20-20		12-14
60	45.0	44-55	40.0	40-40		11-12
84	55.7	40-60	60.0	60-60		9-10
96	77.0	70-90	96.0	96-96		5-7
SPANISH PEANUTS (3 WEEKS OLD)						
0					6.1	5-7
36	0.7	0-3	5.3	0-8	5.2	3.5-6
48	5.0	4-9	70.0	35-90	3.6	3-4.5
84	10.0	5-12	70.5	15-90	3.3	3-4
96	4.0	4.5	88.5	70-100	3.5	2.5-4
120	7.8	6-12	95.8	90-100	2.8	2-4
MANCHU SOY BEANS (4 WEEKS OLD)						
84	0	0-0	0	0-0		
120	0	0-0	0	0-0		
VIRGINIA SOY BEANS (3 WEEKS OLD)						
48	6.2	0-10	4.8	0-10	20.1	12-26.5
72	44.0	25-60	40.0	20-100	17.7	10-23
96	45.0	15-70	39.0	20-80	16.1	10-23
132	51.3	0-70	60.0	20-100	14.4	9-17
TEPARY BEANS (2 WEEKS OLD)						
48	5.0	5-5	0	0-0		
84	80.0	80-80	5.0	5-5		

TABLE 2.—*The effect of chilling at 0.5° to 5° C. on sorghums and maize*

Length of time chilled at 0.5° to 5° C. (hours)	Injury to plants				Height of plants 4 weeks after chilling	
	24 hours after chilling		2 weeks after chilling			
	Average per plant	Range	Average per plant	Range	Average per plant	Range
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
BLACKHULL KAFIR (5 WEEKS OLD)						
0		0-0			19.6	18-20
24 [a]	0	0-0	3.0	0-5	19.1	18-19
48	0	0-0	4.0	3-5	16.5	15-18
72	0	0-0	4.3	2-6	17.2	15.8-18
BLACKHULL KAFIR (6 WEEKS OLD)						
24 [a]	0	0-0	3.0	5-10		
48 [a]	0	0-0	5.4	5-7		
60	0	0-0	3.0	0-15		
72	0	0-0	10.0	10-10		
KANSAS ORANGE SORGO (6 WEEKS OLD)						
0	0	0-0	0	0-0	18.0	16-21
24 [a]	0	0-0	0	0-0	21.6	16-22
48 [a]	0	0-0	2.2	3-5	19.1	16.5-22
60	0	0-0	5.9	0-10	18.8	16-22
72	0	0-0	5.1	0-12	16.2	15-18
MIDLAND YELLOW DENT MAIZE (6 WEEKS OLD)						
0 [a]	0	0-0	0	0-0	38.3	31-40
21	5.0	5-5	5.0	5-5	0	26-36
48 [a]	10.0	10-10	15.0	15-15	0	22-33
60 [a]	20.0	20-20	20.0	20-20	0	22-31
72 [a]	30.0	30-30	25.0	25-25	28.8	21-37

[a] 5 plants only.

These data make it clearly evident that some plants are very sensitive to chilling, while others are exceedingly resistant. Thus an exposure of 12 hours produced noticeable injury in rice and velvet beans, and 24 hours or longer caused severe injury. (Fig. 4, top.) Early Buff cowpeas were severely injured by an exposure of 60 hours or longer. (Fig. 5, top.) Peanuts, Sudan grass, and Teff grass were slightly injured by a 24-hour exposure, severe injury occurring only when chilled for more than 36 hours. Peanuts reacted differently from the others in this group in that the effects were not apparent until several days after the plants were chilled, due to the fact that the roots and not the tops were injured. (Fig. 5, bottom.) Maize, sorghums, watermelons, and pumpkins were hardier, for after 48 hours exposure they showed only about 10 to 15 per cent injury. The remainder of the plants included in this investigation can be considered as highly resistant to chilling. Thus, buckwheat, Tepary beans and Virginia soy beans were injured only 10 per cent when chilled for 60 hours, and potatoes and sunflowers exhibited no injury when chilled for twice that period. The ability of many of these crops, especially tomatoes, to resist chilling is of special interest in view of their frequent inability to survive subzero temperatures.

TABLE 3.—*The effect of chilling on miscellaneous plants*

Number of plants	Age in weeks	Kind of plant	Time chilled	Temperature	Percentage injury 48 hours after chilling	Height of plants 3 weeks after chilling
			Hours			*Inches*
20	2	Early Prolific rice	12	2° to 4° C.	20	8–12
20	2	do	24	do	42	6
20	2	do	36	do	96	Dead.
20	2	do	48	do	100	Dead.
20	2	do	72	do	100	Dead.
20	2	Honduras rice	12	do	12	8–12
20	2	do	24	do	18	6
20	2	do	36	do	25	Dead.
20	2	do	48	do	100	Dead.
20	2	do	72	do	100	Dead.
20	3	Sudan grass	12	do	10	11–16
20	3	do	24	do	10	11–15
20	3	do	36	do	20	11–15
20	3	do	48	do	35	9–11
20	3	do	72	do	50	7– 9
20	3	Teff grass	12	do	11	12–16
20	3	do	24	do	20	12–16
20	3	do	36	do	15	11–13
20	3	do	48	do	35	9–10
20	3	do	72	do	50	Dead.
6	4	Watermelon	36	0.5° to 5° C	10	
		do	60	do	60	
6	4	Pumpkin	48	do	15	
		do	84	do	55	
12	4	Buckwheat	84	do	5	
6	3	Tomato	36	do	5	
		do	84	do	5	
6	8	do	36	do	5	
		do	120	do	0	
12	4	Potato	84	do	0	
6	4	Sunflower	84	do	0	
		do	120	do	0	
30	3	Flax	84	2° to 4° C	0	
		do	120	do	0	

RELATION OF TEMPERATURE TO INJURY

Several of the plants which were found to be exceptionally sensitive, namely Delfos cotton, velvet beans, and Whippoorwill cowpeas, were subjected to a temperature range of 5° to 10° C. for various lengths of time. The injury sustained by each at the various intervals is given in Table 4. It is evident that severe injury occurred at the higher temperatures, but as would be expected a longer period of exposure was necessary. Whippoorwill cowpeas were especially sensitive at this temperature, being rather severely injured by exposure for 48 hours, whereas Delfos cotton and velvet beans survived exposures of the same period without severe injury. This is of special interest in view of the fact that velvet beans were very sensitive at the lower temperatures. As at the lower temperatures, more damage was apparent two weeks after chilling than 24 hours after. Altogether, the results may be taken to show that injurious effects may be brought on with rather brief exposures at temperatures considerably above the freezing point.

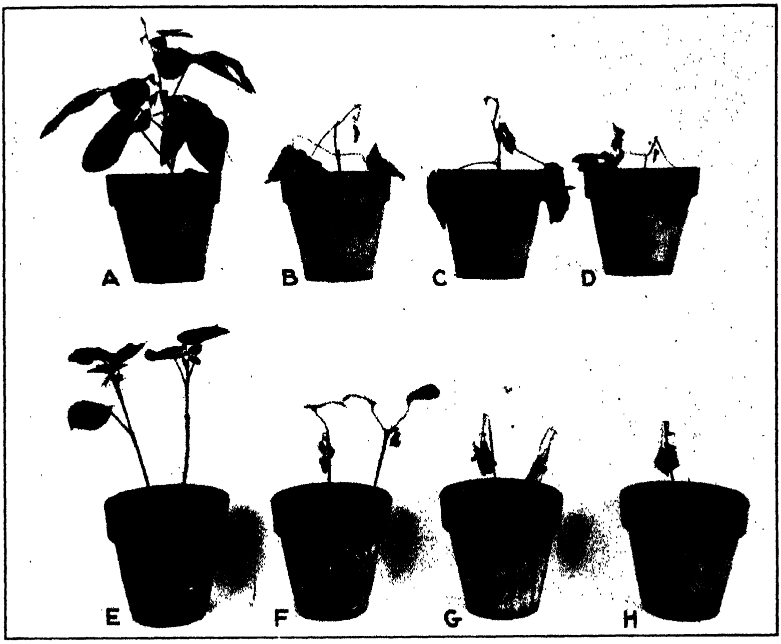


FIG. 4. Injury to velvet beans (top row) and Delfos cotton (bottom row) when plants were chilled at 0.5° to 5° C. A-E, B-F, C-G, and D-H were chilled 12, 36, 60, and 84 hours, respectively

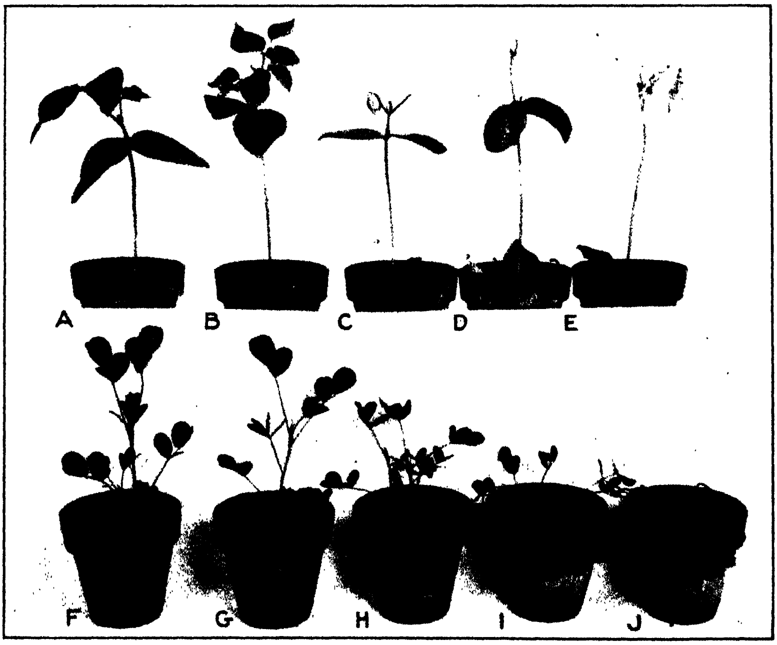


FIG. 5.—Relation of period of exposure to injury caused by chilling 4-week-old Early Buff cowpeas (top row) and 4-week-old Spanish peanuts (bottom row) at 0.5° to 5° C. A-F, B-G, C-H, D-I, and E-J were chilled 0, 36, 48, 84, and 96 hours, respectively

TABLE 4.—*The effect of chilling at 5° to 10° C. on cotton, velvet beans, and cowpea*

Length of time chilled at 5° to 10° C. (hours)	Injury to plants			
	24 hours after chilling		2 weeks after chilling	
	Average per plant	Range	Average per plant	Range
	Per cent	*Per cent*	*Per cent*	*Per cent*
DELFOS COTTON				
48	0	0-0	25.0	25-25
72	3.0	0-12	30.0	30-30
96	7.7	0-12	38.0	35-40
120	51.0	25-60	78.0	40-100
VELVET BEANS				
48	1.8	0-8	1.6	0-6
72	12.2	0-30	9.0	0-30
96	40.0	35-50	42.5	10-100
120	73.0	35-90	86.0	50-100
WHIPPOORWILL COWPEAS				
24	0	0-0	40.0	40-40
48	41.6	20-50	41.6	20-50
72	59.0	35-70	70.0	60-100
96	71.8	65-80	100.0	100
120	92.1	90-96	100.0	100

VARIETAL DIFFERENCES

That there may be important varietal differences has been suggested by the data already presented. In view of the importance of this subject in relation to varietal adaptation and improvement, some tests were made to determine the degree of such differences. Three varieties each of cotton and peanuts were therefore chilled for various periods of time with the results presented in Table 5.

TABLE 5.—*The effect of chilling on varieties of cotton and peanuts*

Variety and age of plants; temperature and duration of chilling	Percentage injury		
	24 hours after chilling	2 weeks after chilling	3 weeks after chilling
Cotton plants (3 weeks old):			
Chilled 24 hours at 2° to 5° C.—			
Trice	60	45	30
Delfos	75	56	40
Westex	65	60	45
Chilled 84 hours at 2° to 8° C.—			
Trice	55	70	70
Delfos	80	85	80
Westex	90	90	95
Chilled 96 hours at 5° to 10° C.—			
Trice	40	70	55
Delfos	75	85	80
Westex	85	95	100
Chilled 108 hours at 5° to 10° C.—			
Trice	60	75	30
Delfos	75	75	70
Westex	85	85	85
Average of 4 exposures—			
Trice	54	65	46
Delfos	76	75	67
Westex	81	82	81
Peanut plants (5 weeks old):			
Chilled 84 hours at 0.5° to 5° C.—			
Virginia Bunch	15	25	12
Spanish	54	88	100
Valencia [a]	68	95	100
Peanut plants (8 weeks old):			
Chilled 144 hours at 2° to 10° C.—			
Virginia Bunch [a]	43	90	
Spanish [a]	60	95	
Valencia [a]	71	100	

[a] 5 plants only.

It is very evident that varieties of cotton and peanuts differ greatly in their ability to resist the influences of chilling. Trice cotton proved to be most hardy followed by Delfos, while Westex was the most sensitive. Thus an average for all trials was 46 per cent injury for Trice, 67 per cent for Delfos, and 81 per cent for Westex. (Fig. 6, bottom.)

But even greater differences were exhibited between varieties of peanuts. Virginia Bunch was exceptionally hardy, while Valencia and Spanish were very sensitive. Thus an exposure of 84 hours was fatal to Valencia but scarcely injured the Virginia Bunch. Spanish was intermediate. The same relation obtained with an exposure of 144 hours. (Fig. 6, top.)

Attention has already been called (Tables 1 to 4) to the susceptibility of Whippoorwill cowpeas, a variety from Georgia which was injured far more than Early Buff from Kansas, although exposed to a temperature approximately 5° higher.

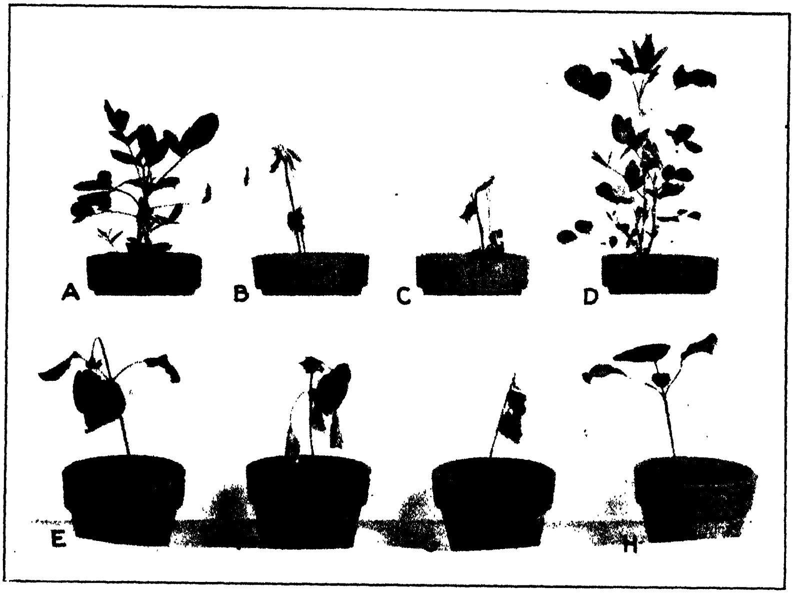


FIG. 6.—The effect of chilling on typical plants of varieties of peanuts (top) and cotton (bottom). Left, to right, top row, peanut plants chilled at 05° to 5° C. for 120 hours: A, Virginia Bunch; B, Spanish; C, Valencia; and D, Spanish peanuts not chilled. Botton row, cotton plants chilled at 2° to 8° C. for 84 hours: E, Trice; F, Delfos; G, Westex, and H, Delfos cotton not chilled

Important differences were also observed in rice, Honduras being injured decidedly less than Early Prolific. (Table 3.) Similar differences were observed with soy beans. Virginia soy beans were severely injured by an exposure of 72 hours, whereas Manchu survived an exposure of 120 hours without injury.

RELATIVE INJURY TO DIFFERENT CROPS

The plants included in this investigation can be conveniently arranged into five classes according to their manner of reacting to low temperatures. This classification is based on the degree of injury as well as on the particular mode of reaction exhibited by each species. Such a classification must of course be considered as distinctly preliminary, owing to the incompleteness of the investigation, and is presented only for convenience.

In class 1 are included those plants which are killed by an exposure of 60 hours to temperatures from 0.5° to 5° C. They are rice, velvet beans, cowpeas, and cotton.

In class 2 are included those which are decidedly injured by such temperatures but which with favorable conditions will recover. This class includes Sudan grass, Teff grass, Spanish and Valencia peanuts.

Class 3 includes those which in general are not likely to suffer serious injury by the conditions specified above. They are Virginia Bunch peanuts, maize, sorghum, watermelons, and pumpkins.

Class 4 includes those which are noticeably injured by prolonged chilling, but in which injury is likely to be nominal, namely, buckwheat, Tepary beans, and soy beans.

Class 5 includes those plants which when exposed at 0.5° to 5° C. were not injured so far as could be observed. They are potatoes, sunflowers, tomatoes, and flax.

THE RELATION OF MOISTURE TO CHILLING INJURY

In order to learn what effect the amount of moisture in the soil had on the percentage of injury, various amounts of water were added for five days before the plants were chilled. Water was withheld 24 hours prior to the application of any treatment. The normal amount of water the plants received each day was 35 to 40 c. c. This was sufficient to keep the soil in each 4-inch pot more or less saturated. The least amount applied was 10 c. c. per pot per day, this being the minimum with which plants could be kept alive. In all, the following amounts were applied in various experiments, namely, 10, 15, 20, 23, 40, 55, 90, 120 c. c. daily and in addition 90 c. c. daily with six hours submersion immediately before chilling. The results as given in table 6 show that the amount of moisture in the soil had a marked effect on chilling injury.

In almost every instance plants were far more severely injured in saturated than in moderately wet soil. In very dry soil they suffered slightly more than in moderately dry, but considerably less than in saturated soil. Thus the injury two weeks after chilling to Velvet beans exposed for 18 hours was 14 per cent in dry soil and 80 per cent in wet soil. Corresponding figures for Early Buff cowpeas exposed for 36 hours were 20 per cent and 40 per cent, and for Spanish peanuts exposed for 84 hours 20 per cent and 75 per cent, respectively. Manchu soy beans were injured slightly more in dry soil, but this may possibly have been due to drought. Cowpea plants were very slow to recover when they were chilled in dry soil, even though they showed here the least reaction; when chilled in saturated soil they recovered very quickly considering the degree of injury.

These observations seem to be in line with the common practice of hardening garden plants by drying prior to transplanting.

The influence of various amounts of moisture in determining the nature of the reaction suggests that the contention often advanced that plants are more severely injured by low temperature during wet weather is by no means amiss; also, that when low temperatures occur in dry weather the effect of chilling is likely to be less severe than if the weather were wet.

TABLE 6.—*The relation of soil moisture to the effect of chilling on various plants*

Plant variety	Chilling temperature	Amount of water applied to plants	Injury to plants				Height of plants	
			24 hours after chilling		2 weeks after chilling			
			Average per plant	Range	Average per plant	Range	Average per plant	Range
	° C.	C. c.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
Velvet beans (3 weeks old).	84 hours at 0.5° to 5°.	15	44	20-60	Died.	Died.	--------	--------
		23	58	20-75	Died.	Died.	--------	--------
		55	68	25-95	Died.	Died.	--------	--------
		90	85	70-94	Died.	Died.	--------	--------
Velvet beans	18 hours at 2° to 4° C.	20	75	0-20	14	5-20	--------	--------
		40	32	10-85	38	15-100	--------	--------
		120	83	65-100	80	60-100	--------	--------
Manchu soy-beans (4 weeks old).	84 hours at 0.5° to 5° C.	15	12.4	10-20	13.0	5-20	15.7	11-19
		23	7.0	7-7	2.6	2-3	20.0	17-24
		55	2.0	1-1	1.4	0.3	25.4	22-28
		90	1.2	1-2	4.6	4-6	27.4	22-31
		[a] 90	0	0-0	.0	0-0	24.0	17-22
Black Eye cowpeas (4 weeks old).	84 hours at 0.5° to 6° C.	15	46	40-50	49	45-50	6.8	6-7
		23	50	40-60	80	80-80	6.6	5-8
		55	60	25-80	86	75-90	9.2	10-11
		90	80	75-90	80	80-80	10.4	10-11
		[a] 90	79	50-80	80	80-85	8.8	7-9
Early Buff cowpeas (3 weeks old).	36 hours at 2° to 4° C.	10	30	30-30	20	--------	8.2	6-9
		20	40	40-40	30	--------	8.5	7-10
		40	40	40-40	40	--------	8.5	7.5-10
Delfos cotton (3 weeks old).	18 hours at 2° to 5° C.	10	2.2	0-5	5	5-5	6.1	6-7
		20	3.0	0-10	10	10-10	5.6	5.5-6
		40	11.8	0-25	20	20-20	6.3	5.5-7
Spanish peanuts (3 weeks old).	60 hours at 2° to 4° C.	10	0	0	7	5-12	3.5	2-5
		20	0	0	12	0-20	4.4	2-5
		40	0	0	25	25-25	4.7	3-5
Spanish peanuts	84 hours at 2° to 4° C.	10	0	0	20	20-20	--------	--------
		20	0	0	60	60-60	--------	--------
		10	0	0	75	75-75	--------	--------
Maize (Kansas sunflower F_1 hybrid (3 weeks old).	36 hours at 2° to 5° C.	10	0	0	6	5-10	9.5	9-10
		20	0	0	35	35-35	10.3	9-12
		40	0	0	50	50-50	9.5	9-10
Maize (Kansas sunflower F_1 hybrid.	60 hours at 2° to 5° C.	10	0	0	7	5-15	--------	--------
		20	0	0	42	40-45	--------	--------
		10	0	0	50	50-50	--------	--------

[a] Submerged for 6 hours prior to chilling.

INFLUENCE OF CHILLING ON PLANTS OF DIFFERENT AGES

In the greater part of this investigation plants were chilled when 3 weeks old. In order to determine the relative sensitiveness of plants at different ages, velvet beans, Delfos cotton, and Black Eye cowpeas of various ages were chilled with the results as given in Table 7.

This experiment shows that in general the younger the plants, the more they were injured. Velvet beans, 28 days old, chilled for 24 hours at 0.5° to 5° C. were injured only 9.4 per cent, while plants half this age showed double the amount of injury and 9-day-old plants were killed. Similar though much less marked differences were observed with the Black Eye cowpeas. As for the cotton plants there was a tendency for them to be most hardy when 35 days old. Even as short a chill as 24 hours at 2° to 4° C. proved fatal to many 10-day-old plants, whereas 17- and 25-day-old ones succumbed only after 48 hours. (Fig. 7.) However, plants 58 days old withered, dropped their leaves, and were injured in all cases more than those 35 days old.

That young plants and the newer parts of old plants suffered more than the full-grown plants and mature parts seems not unnatural. It would follow from this that the changes which ultimately have a detrimental influence occur where the most vigorous growth is taking place, and where cell activity is most intense.

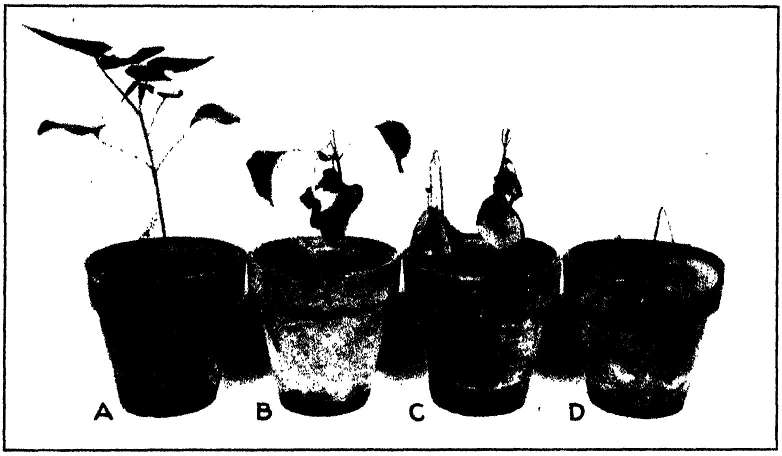


FIG. 7.—Injury to Delfos cotton plants of various ages caused by chilling at 1.5° to 5° C. for 96 hours Left to right: A, 26 days old, not chilled; B, C, and D, 26, 19, and 10 days old, respectively, photographed 24 hours after chilling

TABLE 7.—*The effect of chilling on plants of different ages*

Plant variety	Chilling temperature	Age of plants	Injury to plants: 24 hours after chilling: Average per plant	Injury to plants: 24 hours after chilling: Range	Injury to plants: 2 weeks after chilling: Average per plant	Injury to plants: 2 weeks after chilling: Range
	° C.	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Velvet beans	24 hours at 0.5° to 5°	9	51	30-85		100
		14	20	15-30		80-100
		28	9.4	5-25		70-100
Do	36 hours	9	61	50-85		100
		14	25	20-30		80-100
		28	18	10-30		70-100
Black Eye cowpeas	72 hours at 0.5° to 5°	21	65			
		28	50			
Delfos cotton	24 hours at 2° to 4°	10	21	0-80	21	0-80
		17	10	0-20	10	0-20
Do	48 hours	10	75	60-90	95	90-100
		17	62	10-90	86	40-100
		25	47	10-92	53	5-100
Do	72 hours	10	97	85-100	100	100
		17	96	89-97	100	100
		25	83	65-90	98	90-100
		35	6.6	0-20	9.8	0-30
		58	8.3	4-15	15.3	6-20
Do	96 hours	25	88	70-96	100	100
		35	18.2	0-35	18.1	0-35
		58	62	50-80	71.2	30-100
Do	108 hours	35	30.6	25-50	48	40-60
		58	72.0	70-85	93.7	5-100

THE RELATION OF SALT SOLUTIONS TO CHILLING INJURY

To learn whether the presence of certain salt solutions in the soil would have an influence on the reaction of plants to chilling, the following experiment was conducted.

Forty cubic centimeters of one-twentieth molecular solutions of calcium chloride, calcium nitrate, potassium chloride, potassium nitrate, sodium chloride, and sodium nitrate were applied to peanut, cowpea, maize, and cotton plants for six consecutive days prior to chilling, previous to which they received no water for 48 hours. To the control plants an equal amount of tap water was applied for the same length of time. For the salt solutions tap water was also used. The plants were chilled when 3 weeks old unless otherwise stated. To find out whether base exchange had a possible bearing on the influence of these salts a part of the maize plants were grown in quartz sand with potassium nitrate, calcium nitrate, and tap water. The influence of these solutions on the retardation or hastening of the ill effects of chilling proved very interesting. The results are given in Tables 8 to 12.

TABLE 8.—*The effect of various salt solutions on the reaction of maize to chilling*

BLOODY BUTCHER CORN (4 WEEKS OLD)

Salt solution used	Percentage injury				Height of plants			
	48 hours after chilling		2 weeks after chilling		Plants chilled		Plants not chilled	
	Average per plant	Range	Average per plant	Range	Average per plant	Range	Average per plant	Range
Chilled 72 hours at 0.5° to 5° C.:					*Inches*	*Inches*	*Inches*	*Inches*
Water	51	45-60	26	20- 30	18.4	18-19		
KNO_3	12	10-15	0	0- 0	22.3	22-23		
$Ca(NO_3)^2$	4	3- 8	11	0- 20	25.0	25-25		
$NaNO_3$	11	10-15	19	15 30	19.0	16-21		
Chilled 108 hours at 0.5° to 5° C.:								
Water	20	20-20	78	45-100	17.5		23.9	23.5-24
KNO_3	42	30-60	61	35-100	20.5			
$Ca(NO_3)^2$	46	30-60	86	35-100	20.0			
$NaNO_3$	40	40-40	95	75-100	11.0			

KANSAS SUNFLOWER CORN F_1 HYBRID (2 WEEKS OLD)

Salt solution used	48 hours after chilling: Average per plant	48 hours after chilling: Range	2 weeks after chilling: Average per plant	2 weeks after chilling: Range	Plants chilled: Average per plant	Plants chilled: Range	Plants not chilled: Average per plant	Plants not chilled: Range
Chilled 36 hours at 2° to 4° C.:								
Water			50	50- 50	10	10-10	11.0	11-11
KNO_3			36	30- 40	7.9	7- 8	11.5	11-12
KCl			45.5	45- 48	8.9	8- 9.5	12.0	12-12
CaCl2			60	60- 60	9.0	9- 9	11.0	11-11
$Ca(NO_3)^2$			70	70- 70	8.3	7.5-10	12.0	11-13
NaCl			65	65- 65	9.7	9.5-10	10.0	8-12
$NaNO_3$			84	80- 90	8.0	6- 9	12.0	12-12
Chilled 60 hours at 2° to 4° C.:								
Water			50.0	50- 50				
KNO_3			29.0	25- 45				
KCl			40.0	35- 45				
$CaCl_2$			57.0	57- 57				
$Ca(NO_3)^2$			64.7	61- 68				
NaCl			77.8	70- 90				
$NaNO_3$			85.0	80- 90				

TABLE 9.—*Effect of salt solutions on the reaction of Midland Yellow Dent maize to chilling*

[2-week-old plants grown in quartz sand, chilled 72 hours at 2° to 4° C.]

Salt solution used	Percentage injury after 1 week	
	Average	Range
Water	26.5	20-30
KNO_3	22.2	0-50
Ca (NO_3) [2]	74.3	70-85

TABLE 10.—*The effect of salt solutions on the reaction of Early Buff cowpeas to chilling*

[Plants 3 weeks old; chilled at 2° to 4° C.]

Salt solution used	Percentage injury				Height of plants 3 weeks after chilling			
	24 hours after chilling		2 weeks after chilling		Plants chilled		Plants not chilled	
	Average per plant	Range	Average per plant	Range	Average per plant	Range	Average per plant	Range
Chilled 24 hours:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*	*Inches*	*Inches*
Water	5	0-15	20	15-25	10.1	9-11	13.2	12-14
KNO_3	13	10-15	12	10-15	10.2	9-12	11.5	10-13
KCl	45	45-45	17	15-20	11.0	9-12	10.0	10-10
$CaCl_2$	32	10-40	37	35-40	10.0	9.5-10.5	12.4	10-14
Ca (NO_3) [2]	49	35-55	32	30-35	10-2	9-13	12.2	11-13
NaCl	40	25-55	42	40-45	10.1	9.5-11	10.5	9.5-11
$NaNO_3$	50	50-50	50	45-55	10.1	9.5-11	12.2	11-13
Chilled 36 hours:								
Water	40	40-40	40	40-40	8.5	7-10	13.2	12-14
KNO_3	40	40-40	50	50-50	8.3	7.5-10	11.5	10-13
KCl	50	50-50	40	40-40	8.5	8-10	10.0	10-10
$CaCl_2$	56	50-60	70	70-70	8.4	7-10	12.4	10-14
Ca (NO_3) [2]	50	50-50	60	60-60	9.0	8-10	12.2	11-13
NaCl	60	60-60	75	75-75	7.7	7-8.5	10.5	9.5-11
$NaNO_3$	65	65-65	85	85-85	7.9	7-8.5	12.2	11-13

TABLE 11.—*The effect of salt solutions on the reaction of Spanish peanuts to chilling*

Salt solution used	Average percentage injury				Average height of plants	
	24 hours after chilling	1 week after chilling	2 weeks after chilling		2 weeks after chilling	
			Entire plant	Roots only	Chilled	Not chilled
Chilled 60 hours 2° to 4° C.:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
Water	0	25	40	60	4.5	5.5
KNO_3	0	50	20	30	4.0	7.0
KCl	0	18	30	40	3.5	7.5
$CaCl_2$	0	18	50	50	3.5	7.5
Ca(NO_2)[2]	0	29	75	75	4.5	6.0
NaCl	0	65	60	55	3.5	7.0
$NaNO_3$	0	75	80	80	4.0	7.0
Chilled 84 hours 2° to 4° C.:						
Water	0	75				
KNO_3	0	75				
KCl	0	80				
$CaCl_2$	0	90				
Ca(NO_3)	0	90				
NaCl	0	80				
$NaNO_3$	0	90				

TABLE 12.—*The effect of salt solutions on the reaction of cotton to chilling*

WESTEX COTTON (4 WEEKS OLD)

Salt solution used	Percentage injury			Height of plant		
	24 hours after chilling		2 weeks after chilling	Chilled		Not chilled
	Average per plant	Range	Average per plant	Average per plant	Range	Average
Chilled 48 hours at 0.5° to 5.0° C.:	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*	*Inches*
Water	17.0	15-40	20	11.4	10.5-12.5	----------
KNO_3	24.0	10-35	10	11.6	11 -12.5	----------
$Ca(NO_3)^2$	35.0	5-70	20	11.8	11 -11.5	----------
$NaNO_3$	22.0	10-30	30	10.0	9 -11	----------

DELFOS COTTON (3 WEEKS OLD)

Salt solution used	Percentage injury			Height of plant		
	24 hours after chilling		2 weeks after chilling	Chilled		Not chilled
	Average per plant	Range	Average per plant	Average per plant	Range	Average
Chilled 18 hours at 2.4° to 4.0° C.:						
Water	11.8	0-25	20	6.3	----------	6.5
KNO_3	17.0	0-25	10	6.3	----------	6.6
$Ca(NO_3)^2$	37.0	30-50	20	6.0		6.0
$NaNO_3$	26.0	15-30	25	6.8	----------	6.5

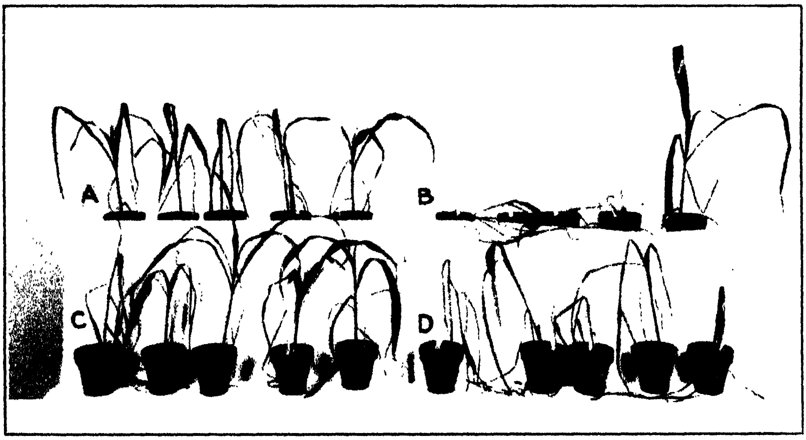


FIG. 8.—Injury to 4-week-old Midland Yellow Dent maize when chilled at 0.5° to 5° C. for 108 hours (severe) after being treated with one-twentieth molecular solution of $Ca(NO_3)^2$ (A), $NaNO_3$ (B), KNO_3 (C) and tap water (D)

The least injury occurred where potassium nitrate had been applied; next in protective effect was potassium chloride. Next in order came those plants that received an equal amount of water, calcium chloride, calcium nitrate, and sodium chloride, while those that had sodium nitrate were injured most, in several instances fatally. (Fig. 8.)

It seems that the potassium cation assisted the plants to withstand chilling, whereas calcium appeared in several instances to cause more injury than that perceptible on the control plants. Sodium had a decidedly deleterious effect. As for the anions less injury occurred where chlorides had been used than with the corresponding nitrates.

The maize plants grown in quartz sand showed that the salt applied rather than a liberated base was responsible for the difference in injury. In this instance the plants treated with potassium nitrate showed slightly less injury than the control plants, while plants that had received calcium nitrate were nearly killed.

The different reactions brought out by the salt solutions may have been due to the effect of the individual salts on the constitution of the plants. As far as is known, all the cations and anions used, including sodium, are taken up by plants. Especially interesting is the fact that potassium assisted the plants to endure chilling, when it is considered that potassium fertilizers are recommended for application to fields where mazie suffers from root rot. In this experiment, those plants that were treated with sodium nitrate, and were incidentally the most severely injured, were the only ones to show outward indications of the presence of a fusarium similar to that causing maize root rot.

CHILLING INJURIES IN NATURE

Observations made at Colby and Fort Hays experiment stations, and in the Union of South Africa prove that the inimical effects of chilling in nature are in no way different from those artificially produced.

On June 19, 1927, at Colby, Kans., cotton plants 1 month old were observed to have been injured and many killed by "frost." But for at least four weeks prior to this date no frost had been recorded. The weather records, however, showed that the maximum temperatures for June 13 and 14 had been 49° and 50° F., respectively, while the minimum for June 12, 13, and 14 were 45°, 43° and 45° F. respectively. In terms of degrees centrigrade there had been at least a 36-hour period during which the temperature did not go above 10°. The last three days mentioned were cloudy and misty and there was a daily precipitation of 0.6, 0.2, and 0.1 inch, respectively, during that time. The cold and wet weather during these three days was undoubtedly responsible for the injury noted above.

At Fort Hays, Kans., Faris bands in more exaggerated form than those artificially produced were observed on sorghums, maize, and Sudan grass on October 1, 1927. They were particularly noticeable on the leaves of young suckers or the secondary growth of exposed plat corners and on young volunteer plants. A cold and misty period from September 25 to 27 was held responsible for the abnormalities observed. The maximum temperatures for the 26th and 27th were 43° and 45° F., respectively, while the minimum for the 25th, 26th, 27th, and 28th were 42°, 36°, 37°, and 34° F., respectively. In terms of degrees centigrade, during the 26th and 27th there was a 36-hour period when the temperature did not go above 7°.

After the experiments reported here had been completed, both the chilled and the other plants in the greenhouse showed characteristic chilling injury during a cold period from the 17th to the 22d of September. During this period the temperature remained below 50° F. (10° C.) for 13 hours immediately before and after midnight of September 20. The minimum reached was 39° F. (3.89° C.). Normal 3-week-old cowpea plants three days after this chilling behaved no differently than those that had been artificially chilled for 12 hours at 5° to 10° C.

A. R. Saunders reported that during a cold spell in November, 1927, at the experiment station at Potchefstroom, Union of South Africa early-planted maize and amber cane exhibited prominent Faris bands.[4]

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[4] Private correspondence; letter of November 26, 1927.

GENERAL DISCUSSION

The investigations reported herein point definitely to the fact that chilling initiates certain changes in crop plants which have a detrimental influence on them. The results thus agree almost completely with those obtained by Molisch (*12*), Faris (*5*), and Pantanelli (*15, 16, 17*). It is of further interest to note that most of the characteristic abnormalities observed by Molisch were noted also in the present study of crop plants and could be described in more or less the same way as recorded by him.

The relative sensitiveness of the plants included in this investigation suggests that their distribution may be dependent quite as much upon their ability to endure chilling as upon other climatic factors, such as total heat units, mean temperatures, frost-free periods, etc., which ordinarilly receive much more attention. Thus plants that proved most sensitive to chilling are the staple crops of the subtropics, such as rice, velvet beans, cotton, and peanuts, while the hardier ones are extensively grown in temperate regions, as, for example, maize, sorghums, watermelons and pumpkins. The most hardy have a very wide distribution, but are essentially northern annuals, such as soy beans, buckwheat, flax, and sunflowers.

That varietal distribution may also be dependent upon chilling injury is definitely suggested by the difference in hardiness of certain varieties of cotton, peanuts, cowpeas, and soy beans.

What the ultimate cause of the deleterious influences that follow chilling may be is a matter requiring further investigation. It is well known that physiological processes are definitely limited by temperature, some much more than others. Puriewitsch (*18*), according to Palladin (*14*), found that the respiratory ratio is at a minimum from 2° to 4° C.; that is, at this temperature oxygen absorption proceeds much faster than the elimination of carbon dioxide. Oxidation in the cells is therefore not complete and toxic substances may be formed. It is therefore conceivable or even probable that low temperatures upset the sensitive balance between the various processes with the result that harmful products accumulate in the plant cells. This seems to have been Molisch's view (*12*).

In the investigations reported in this paper greater injury was observed on plants growing in saturated, and therefore in an oxygen-deficient soil, than in dry soil. This conceivably might accentuate the relation noted above.

The carbohydrate nitrogen ratio in plants may also be a very important factor in determining the resistance to chilling. Pantanelli concluded from his experiments that those plants which retained the most sugar are able to withstand cold best, because at low temperatures carbohydrates are used in respiratory combustion. He pointed out further that as long as a cell has sugar at its disposal it does not consume albumens and when the sugar is exhausted autodigestion of the proteins takes place. Since nitrogen is a limiting factor in growth, it follows that the more nitrogen there is present in the plant the more carbohydrates are used up in building new tissue. According to the work of Fischer (*6*) and Kraus and Kraybill (*9*) a rapid vegetative growth is characterized by a high nitrogen carbohydrate ratio. Hence those plants making the most active growth and thus using considerable carbohydrates are likely to have the least in reserve for respiratory combustion during a cold period. This hypothesis is substantiated by the fact that in general where nitrates were applied the greatest amount of injury took place. The single exception, with potassium nitrate, is easily explained by the well-known fact that potassium functions especially in photosynthesis and in the movement of carbohydrates within the plant, and that it tends to counteract the ill effects of too much nitrogen.

Nelson (*13*) suggests from his own and the work of Armstrong (*1*) that at low temperatures there is a liberation or accumulation of some toxic fragments resulting from the mixing of hydrolytic enzymes and glucoside. The protoplasm being unable to function properly at the low temperatures can not throw off these toxic compounds in the normal manner.

That certain plants die from drought after their roots have been injured to a greater or lesser extent is most probable. In the present study this was found to be true with peanuts. The plants did not show injury or perish until the roots had been injured to such an extent as to be unable to supply the top growth with an adequate amount of water.

In conclusion it must be pointed out that the chilling of plants should not be considered from the point of air temperatures alone; soil temperatures warrant as much consideration. In spring soil temperatures are considerably lower than air temperatures and the former fluctuate far less than the latter. The top growth of the plants may experience a chill first, and when the air has warmed up again the roots may still be in very cold soil. The chilling period to which the plant as a whole is subjected may thus be longer than that indicated by either soil or air temperatures separately.

SUMMARY AND CONCLUSIONS

The results of the experimental work reported in this paper support the following conclusions:

In most summer crop plants constitutional disturbances having a serious influence on them are called into effect when the plants are subjected to chilling, even though the temperature does not go as low as 0°C.

The outstanding result of the investigation was the very evident effects of chilling on certain plants and the high degree of resistance in others. The duration and the intensity of the cold were important factors in determining the nature of the reactions. An exposure of 24 and 36 hours at 0.5 to 5.0° C. was fatal to rice, velvet beans, and cotton. With the same exposure cowpeas were completely defoliated and only straggly plants were produced. Peanuts, Sudan grass, and Teff grass exposed to chilling temperatures for 48 hours appeared at first to be uninjured, but died in the course of about two weeks; at the same exposure maize, sorghums, watermelons, and pumpkins were slightly injured. Soy beans, potatoes, buckwheat, Tepary beans, tomatoes, and flax proved to be exceedingly hardy, it being possible to expose them to chilling temperatures for 84 to 96 hours without injury. At 5° to 10° C. velvet beans proved to be hardier than Whippoorwill cowpeas. At this temperature cowpeas were injured when exposed for 24 hours, velvet beans and cotton when exposed 60 hours.

Marked differences in relative hardiness between varieties was observed. Trice cotton, which is grown near the northern limit of the Cotton Belt in North Carolina, proved more hardy than Delfos from Mississippi, while Westex, a variety specially bred for Texas conditions, was considerably more tender than either of the foregoing. Whippoorwill cowpeas from Georgia became chlorotic when chilled for 12 hours at 5° to 10° C., whereas Early Buff from Kansas did not assume this condition until chilled for 36 hours at 0.5° to 3.0°. Virginia Bunch peanuts were hardier than Spanish and the latter hardier than Valencia. Early Prolific rice was more sensitive than Honduras, and Manchu soy beans were hardier than Virginia. The relative hardiness of the different plants indicates that their distribution from subtropical to temperate regions may be dependent to some extent upon their ability to endure chilling.

A point of considerable interest was the characteristic reactions of different species, not only with respect to differences in degree of injury, but also with respect to the nature of the injury. Cowpeas and rice leaves became chlorotic 24 to 48 hours after chilling, while cotton and soy beans showed brown and white spots, respectively, and the grasses in general showed white bands across the leaves similar to those described by Faris in sugar cane. Lesions were observed on field crops at Hays and Colby and at Manhattan on greenhouse plants when the air temperature remained between 33° and 50° F. for one to three days.

Cowpeas, peanuts, maize, and velvet beans were far more severely injured in wet than in dry soil. Soy beans behaved in the contrary manner, although the injury in the latter case was slight and may possibly have been due to drought.

In experiments with cowpeas, cotton, and velvet beans, young plants suffered materially more than old plants treated in a similar manner.

Tap water solutions of various salts were applied to the plants to ascertain whether any salts would act protectively against plant injury by chilling. Equal volumes of tap water were applied to control plants. Potassium nitrate was found to afford considerable protection, with potassium chloride standing next in order. Then came the control lot, and then, in descending order of effectiveness, the lots to which calcium chloride, calcium nitrate, sodium chloride, and sodium nitrate were applied. Sodium nitrate and sodium chloride were in some cases fatally injurious when chilling was continued for from 36 to 60 hours, the former salt being the more injurious.

It appears that the potassium cation assisted the plants to withstand chilling better than if they were merely moistened with tap water. Calcium, however, seemed to exert no such protective influence, as plants receiving it were in some instances injured more by chilling than the control plants. Sodium had a decidedly deleterious effect. As for the anions, less injury seemed to occur in the presence of the chlorides than when nitrates were used, except in the case of potassium nitrate.

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INJURY TO ONIONS AND FRUITS CAUSED BY EXPOSURE TO AMMONIA[1]

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INTRODUCTION

For several years the pathologists of the Bureau of Plant Industry associated with the food-products inspection service of the Bureau of Agricultural Economics have been asked from time to time to explain the cause of certain brownish and greenish black discolorations of fruits and onions. In most cases the products in question were free from disease and appeared perfectly normal in every respect except color. The discoloration of onions has been observed more frequently than that of any other product, but similar discolorations of apples, pears, bananas, and peaches have been seen.

RESULTS OF INVESTIGATIONS

ONIONS

The discoloration of onions was investigated first. The senior writer was asked to decide whether a certain lot of onions had been injured in storage. The grower claimed that the onions were bright yellow, clean, and in good condition when they were placed in cold storage, but that when they were taken out to be placed upon the market the outer dry scales were brownish black, as if they had been scorched or burned. Careful examination of the samples furnished showed that the fleshy scales were in good condition and normal in color. No indication of freezing injury or parasitic disease could be found, consequently it became necessary to look for other causes of the discoloration. The fact that the injury in this particular lot of onions appeared to be directly associated with some unusual storage condition suggested the possibility that escaping ammonia from the cold-storage system might have been the causal agent.

In order to study the effect of ammonia fumes on onions, several white onions were placed on wire racks in two 5-liter jars, 15 c. c. of ammonia (specific gravity 0.90) was poured into each jar, and a glass plate was sealed over the top of each with vaseline. These experiments were identical except that the onions in one jar were moistened with water, while those in the other were dry. Within an hour the moistened onions were slightly yellowish, while the dry ones were only faintly greenish yellow along the broken edges of the dry scales. These onions were kept under observation in the ammonia fumes for one month. At the end of this period the moistened onions were

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"chamois" [3] to light brown and the dry ones had a metallic sheen and were "cinnamon buff" in color. This prolonged exposure caused the death of all tissues and a yellow to mustard-yellow discoloration throughout all bulbs. However, all the badly discolored onions observed on the market were yellow, brown, or red varieties.

Yellow onions, when treated like the white ones, immediately began to turn greenish yellow and then brown. After an exposure of one hour all of them were dark, some deep sepia brown, and others almost black. The moistened onions darkened first, but there was little difference between the two lots at the end of an hour. They were kept under observation in the ammonia fumes for three days. At the end of this period all of them were dark brown to metallic black. The brownish black discoloration was confined to the pigment-bearing outer dry scales, but there was also much injury to the two or three outer layers of fleshy scales, which were soft, flabby, and greenish yellow.

These preliminary experiments showed that strong ammonia injures onions seriously, and indicated that ammonia had probably caused the discoloration of the storage onions that led to this investigation. The results served as a basis for further work on the discoloration of onions and on certain similar discolorations of fruits.

Additional tests were made in the laboratory for the purpose of checking preliminary experiments and observations made on the market and of studying the progressive action of known concentrations of ammonia on the plant tissues and the subsequent reactions of the specimens. No attempt was made to determine the minimum quantity of ammonia that would cause injury. Although no critically quantitative tests were made, the percentage of ammonia in the air was determined. This was done in two ways: First, by means of a gas burette; [4] and second, by titrating solutions of ammonium hydroxide and calculating from the molar concentration the percentage of ammonia in the solution. Two strengths of ammonium hydroxide were used. The normality, the percentage of ammonia, the resulting vapor pressure [5] of these, and the percentage of ammonia in the air are shown in Table 1. The tests with this method were carried out at a temperature of 70° F. and a barometric pressure of 750.7 mm. Equal quantities of each of the solutions were placed in dishes under bell jars; then, after 10 minutes, to insure diffusion of the ammonia through the air, the specimens were supported on wire baskets over the solutions and the observations were begun.

TABLE 1.—*Significant figures in the calculation of percentages of ammonia in the air from solutions of ammonium hydroxide*

Normality of solution	Ammonia in solution (per cent by weight)	Vapor pressure of ammonia (mm. mercury)	Ammonia in air (per cent by volume)
2.68	4.56	30.40	4.18
11.30	19.25	212.90	29.29

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[3] Throughout this paper all quoted color names are from the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

[4] All determinations were made by means of the gas burette except the experiments at 70° F. with 4.2 and 29.3 per cent ammonia.

[5] LANDOLT, H. H. LANDOLT-BÖRNSTEIN PHYSIKALISCH-CHEMISCHE TABELLEN. Ed. 5, p. 1397. Berlin. 1923.

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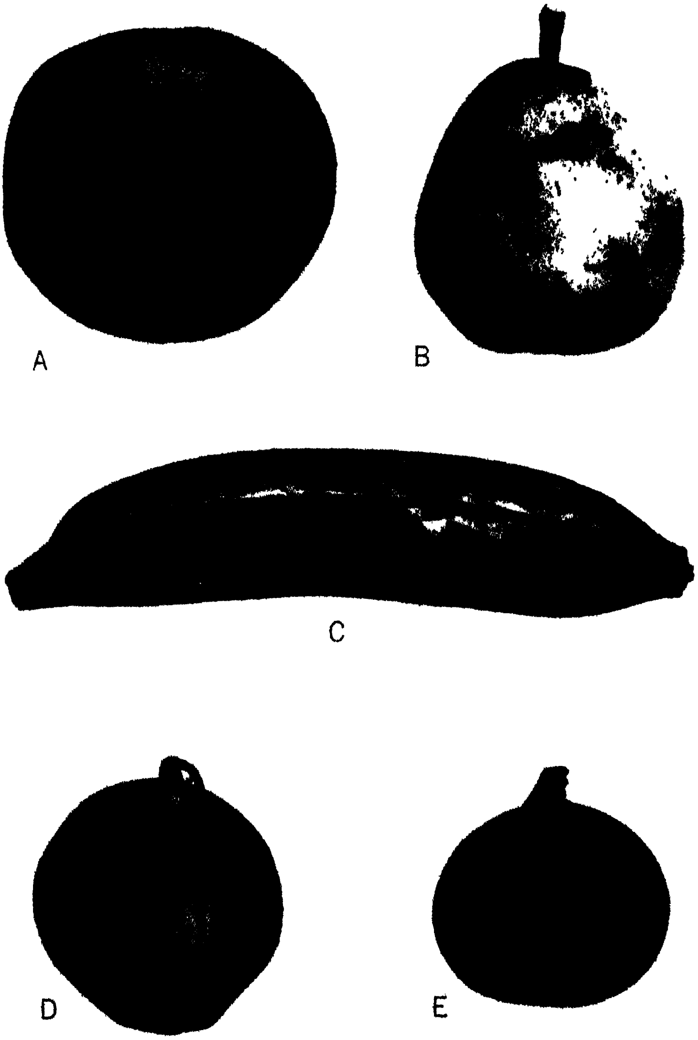

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Ammonia injury of, A, Jonathan apple; B, Winter Nelis pear; C, banana, D, Yellow Globe onion; E, Red Globe onion.

In Table 2 are given the results of representative experiments conducted at a temperature of 70° F. and at relative humidities of 30 and 85 per cent, in which yellow and red onions were exposed to known strengths of ammonia for definite periods of time. The color changes are described in each instance. It will be noted that the edges of the dry color-bearing scales began to show a greenish yellow discoloration almost instantly in each of the concentrations of ammonia used. This color change is characteristic and marks the initial reaction which eventually results in various gradations of color from greenish yellow in white onions to brown in yellow ones (pl. 1, D) and deep greenish black in red ones (pl. 1, E), upon prolonged exposure to ammonia. The results of these experiments indicate that a small amount of ammonia (0.8 per cent) in the air will cause in a lesser degree the same discoloration that is produced by a high percentage, but that the rate will be slightly lower. This is especially noticeable with the yellow onions, which as a rule are somewhat slower than the red ones in showing the effect of ammonia. The character and condition of the pigments as well as the degree of pigmentation determine the extent of discoloration possible. In all experiments the most highly colored specimens showed the most injury.

TABLE 2.—*Effect of ammonia on Red Globe and Yellow Globe onions at a temperature of 70° F., and relative humidities of 30 and 85 per cent*

Relative humidity (per cent)	Percentage of ammonia	Duration of exposure	Color changes in—	
			Red onions	Yellow onions
30	0.8	½ minute	Greenish (“citron yellow” to “yellowish citrine”) along edges of scales.	No change.
		2 minutes	—	Yellowish green (“citron yellow”) along edges of scales.
		10 minutes	Large blackish green (“dull blackish green”) blotches prominent.	Light-bronze (“medal bronze”) blotches prominent.
		42 hours	50 per cent of surface blackish green (“dull blackish green”).	20 per cent of surface bronze (“medal bronze” to “raw umber”).
30	1.0	½ minute	Blackish green (“dull blackish green”) blotches prominent.	Edges of scales greenish brown (“dark citrine”).
		15 minutes	Large blackish green (“dull blackish green”) blotches.	Large bronze (“medal bronze”) blotches.
		30 minutes	70 per cent of surface blackish green (“dull blackish green”).	Large dark bronze (“medal bronze” to “raw umber”) blotches.
		48 hours	90 per cent of surface dark greenish black (“dull greenish black (2)”).	75 per cent of surface dark bronze (“raw umber”).
85	4.2	½ minute	Blackish green (“dull blackish green”) along edges of scales.	Greenish yellow (“yellowish citrine”) along edges of scales.
		30 minutes	Large dark blotches of blackish green (“dull blackish green”).	Cinnamon brown (“cinnamon rufous”) in blotches.
		1½ hours	Most of surface dark greenish black (“dull greenish black (2)”).	Large cinnamon-brown to brownish black (“cinnamon-rufous” to “chestnut brown”) blotches.
		4½ hours	95 per cent of surface dark greenish black (“dull greenish black (2)”).	95 per cent of surface bronze to brownish black (“Chestnut brown” to “Vandyke brown”).
85	29.3	½ minute	Blackish green (“dull blackish green”) along edges of scales.	Greenish yellow (“yellowish citrine”) along edges of scales.
		30 minutes	Most of surface blackish green (“dull blackish green”).	Most of surface brown (“cinnamon brown”).
		1½ hours	90 per cent of surface dark greenish black (“dull greenish black.”)	Most of surface dark brown (“chestnut brown”).
		4½ hours	98 per cent of surface dark greenish black to metallic black (“dull greenish black (2)”).	98 per cent of surface blackish brown (“Vandyke brown”).

Since the discoloration took place so rapidly at 70° F., it seemed advisable to conduct experiments to determine what effect low temperatures would have upon the rate of discoloration. Consequently, several experiments were made with comparatively low percentages of ammonia in a cold-storage room where the temperature was 31.5° and the relative humidity 83 per cent.

The results of typical experiments conducted under low-temperature conditions are shown in Table 3. The characteristic greenish black discoloration of the red onions and the yellowish green and brown of the yellow ones were quite noticeable within a few minutes in each of the concentrations. With 0.8 per cent ammonia the initial color changes took place somewhat more slowly than in the corresponding experiments at 70° F., but marked discoloration was produced in less than an hour. With 3.2 per cent ammonia the greenish discoloration along the broken edges of the dry color-bearing scales was almost instantaneous, prominent blotches being evident within one-half minute. This action is as rapid as that recorded for higher concentrations of ammonia at 70°. In experiments where concentrations as low as 3.2 per cent were used at both 31.5° and 70° no difference in the initial rate of discoloration could be detected. These experiments, together with others of a similar nature that were conducted in cold storage, show, therefore, that low temperatures do not materially influence the rate of discoloration of onions exposed to ammonia.

Humidity is one of the important factors in determining the rate of discoloration, and with concentrations of ammonia lower than 1 per cent some experiments indicate that it may determine whether or not any discoloration will take place. In all experiments where any noticeable variation in air moisture or dryness of the onion scales existed, discoloration was always much more rapid and pronounced under the more humid conditions.

TABLE 3.—*Effect of ammonia on Red Globe and Yellow Globe onions at a temperature of 31.5° F., and a relative humidity of 83 per cent*

Percentage of ammonia	Duration of exposure	Color changes in—	
		Red onions	Yellow onions
0.8	7 minutes	Edges of broken scales yellowish green ("yellowish citrine").	No change.
	43 minutes	10 per cent of surface blackish green ("dark olive" to "dull blackish green").	Edges of broken scales yellowish green ("citron yellow").
	19½ hours	30 per cent of surface blackish green ("dull blackish green").	Yellowish green to greenish brown ("yellowish citrine" to "dark citrine") along edges of scales and 10 per cent of surface.
2.6	5 minutes	Blackish green blotches evident ("dull blackish green").	Greenish brown blotches prominent, ("dark citrine").
	17½ hours	98 per cent of surface dark greenish black ("dull greenish black (2)").	95 per cent of surface dark bronze ("raw umber" to "chestnut brown").
3.2	½ minute	Blackish green ("dull blackish green") blotches prominent.	Greenish brown ("dark citrine") along edges of scales.
	8 minutes	Most of surface blackish green ("dull blackish green").	Most of surface metallic bronze ("raw umber" to "chestnut brown").
	1½ hours	95 per cent of surface dark greenish black to metallic black ("dull greenish black (2)").	90 per cent of surface bronze to brownish black ("chestnut brown" to "Vandyke brown").

Many of the ammonia-injured onions observed on the market resembled onions exposed experimentally at a temperature of 70° F. and a relative humidity of 85 per cent, to 29.3 per cent ammonia for 1½ hours, and 4.2 per cent ammonia for 4½ hours, or at a temperature of 31.5 ° and a relative humidity of 83 per cent to 2.6 per cent ammonia for 17½ hours. The injury at the end of these exposures is to be classed as a blemish, since the color-bearing dry outer scales are usually the only ones affected. Any of the fleshy scales that are exposed by the removal of the dry protective scales turn greenish yellow within the time limits mentioned above, but if the dry scales are intact, there is no injury of the edible portion of the bulbs. Longer exposures are practically certain to cause some discoloration and softening of the fleshy scales. The reactions of the individual bulbs show wide differences in this respect, however, on account of the variations in number, texture, and condition of the protective scales. When onions are exposed to ammonia long enough to cause death of the outer fleshy scales they become greenish yellow, watery, and unmarketable and subject to attack by decay-producing organisms.

APPLES

When well-colored, firm Jonathan apples were exposed to ammonia fumes they quickly became discolored, the injury showing first around the lenticels. After removal from the fumes the apples began to recover their normal color almost at once, and after 12 hours practically all of the coloring had returned and no further change could be noted. The progressive changes while in contact with the ammonia are recorded in Table 4, in which, for convenience, results with other fruits are also included. As just indicated, the changes represent transitory conditions.

After the recovery noted above no change could be observed and whatever discoloration remained was permanent. The appearance of apples that have been exposed to ammonia is characterized chiefly by the discoloration around the lenticels. (Pl. 1, A.) On the blushed side they are surrounded by dark rings, which are generally from 1 to 2 mm. in diameter and darker than the surrounding tissues. These rings are black when they occur on the deep-red areas and green on the yellow-green areas.

Jonathan apples were also exposed to ammonia (0.8 and 1.0 per cent) at a temperature of 70° F. and a relative humidity of 30 per cent. The rate of discoloration was slightly slower than that recorded for the higher percentages of ammonia (Table 4), but in general the results were not sufficiently different to warrant detailed discussion.

The effect of low temperature upon the discoloration of apples was also determined. (Table 5.) The results indicate that lowering the temperature did not appreciably alter the rate of discoloration.

PEARS

In their work on the storage of pears, Overholzer and Latimer[6] describe ammonia injury of pears as follows:

> Pears contain a substance that becomes dark when alkaline and colorless when acid. Ammonia gas gives an alkaline reaction, and since the fumes are quite soluble in fruit juices, they may be absorbed through the lenticellike openings

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[6] OVERHOLZER, E. L., and LATIMER, L. P. EFFECT OF AMMONIA FUMES UPON PEARS IN COLD STORAGE. Calif. Agr. Expt. Sta. Rpt. 1922/23: 201-203, illus. 1923.

or "dots" of the epidermis sufficiently to bring about an alkaline condition in local areas.

The oxidation processes in pears also proceed more rapidly in a slightly alkaline medium than in the acid tissues of the fruit. Furthermore, the concentration of ammonia may become sufficiently great to act deleteriously by increasing the permeability of the cells and thus disorganizing the protoplasm and permitting the mixing of the oxidizing enzyme and substrate. Hence, fruit subjected to ammonia gas or fumes may also darken by oxidation, but the discoloration thus produced is apparently distinct from that effected immediately by alkalinity. The two colors, however, are inseparable upon the basis of appearance.

Table 4.—*Effect of ammonia on Jonathan apples, Bartlett pears, bananas, and peaches at a temperature of 70° F., and at relative humidity of 85 per cent*

Percentage of ammonia	Duration of exposure	Color changes
		APPLES
4.2	1 minute	Lenticels browned.
	2 minutes	Do.
	6 minutes	Few dark rings around lenticels.
	11 minutes	Few greenish rings around lenticels on yellow side of apple.
	19 minutes	Slight darkening of red areas.
	30 minutes	A few additional rings and streaks of reddish purple ("dark Corinthian purple") through purplish red ("burnt lake"). No discoloration of unblushed areas.
29.3	1 minute	Lenticels browned.
	2 minutes	Dark rings around a few lenticels.
	6 minutes	Rings more numerous, larger and darker.
	11 minutes	Lenticels show white specks in center. Unblushed areas changing to yellowish green.
	19 minutes	Unblushed areas yellowish green. Blushed areas dark, nearly black.
	30 minutes	Deeper red has turned to glossy black that shades through brown and red into greenish yellow ("deep colonial buff") of unblushed areas.
		PEARS
4.2	2 minutes	Lenticels rather prominent, greenish brown.
	7 minutes	Lenticels rather prominent, greenish brown. Minute drops of moisture on the surface.
	12 minutes	Do.
	17 minutes	No change in number of spots but spots changed to dull red ("brick red").
	20 minutes	Surface generally moist, spots reddish brown, no coalescing visible.
29.3	2 minutes	Lenticels prominent dull red ("brick red").
	7 minutes	Increase in size and number of red lenticels, surface quite moist.
	12 minutes	Spots enlarged due to spreading of discoloration from lenticels.
	17 minutes	Spots coalescing rapidly.
	20 minutes	Surface wet. Ground color light brown ("Sanford's brown") with reddish brown ("burnt sienna") blotches.
		BANANAS
4.2	12 minutes	Few small yellowish-brown dots on sides.
	28 minutes	10 per cent of surface spotted as above.
	1 hour	25 per cent of surface brown; ribs dark brown to black. Ends black, some reddish brown blotches.
29.3	4½ minutes	Minute yellowish brown dots generally distributed.
	10 minutes	Spots darker brown, 20 per cent discoloration. Dead-ripe appearance.
	24 minutes	Ribs solid black. Minute drops of moisture on surface.
	33 minutes	Generally dark brown with black ribs and ends.
	1 hour	Uniformly brownish black ("Mars brown" to "clove brown").
		PEACHES
4.2	1 minute	Minute greenish-black specks.
	3 minutes	Red color of peach gone. Many greenish black specks.
	23 minutes	60 per cent of surface dull yellowish green ("olive lake" and "buffy citrine").
	1 hour 40 minutes	Almost solid dull yellowish green ("olive lake").
29.3	1 minute	Minute greenish black specks.
	23 minutes	Dull yellowish green ("olive lake" and "buffy citrine").
	1 hour 10 minutes	Darker with a reddish cast. Some moisture on surface.
	1 hour 40 minutes	As above with large reddish brown blotches and large drops of moisture, on surface.

TABLE 5.—*Effect of ammonia on Jonathan apples, Anjou pears, and bananas at a temperature of 31.5° F., and a relative humidity of 83 per cent*

Percentage of ammonia	Duration of exposure	Color changes
		APPLES
0.8	9 minutes	Few lenticels show dark borders and white centers.
	43 minutes	Many lenticels show heavy black borders and white centers.
	19½ hours	Lenticels prominent as white dots, but few black borders remain.
2.6	5 minutes	Lenticels prominent, black borders with white centers.
	17½ hours	Lenticels white surrounded by black rings 1.5 to 4 mm. in diameter.
3.2	½ minute	Lenticels prominent, few black borders.
	8 minutes	Lenticels prominent with black rings, discoloration spreading between rings.
	1½ hours	90 per cent of surface discolored. Lenticels with white centers and black borders.
		PEARS
0.8	22 minutes	Lenticels slightly watery. Light reddish blotches appearing.
	43 minutes	Lenticels more moist and reddish blotches more prominent.
	19½ hours	Lenticels watery, blotches as above.
2.6	5 minutes	Few slightly reddish blotches.
	17½ hours	Few slightly reddish blotches. Few lenticels with reddish borders, fruit deeper yellow and more mature looking.
3.2	2 minutes	Few light reddish blotches.
	8 minutes	Few reddish brown blotches.
	1½ hours	Reddish brown blotches larger. Ruptures in skin reddish brown.
		BANANAS
0.8	9 minutes	Few brownish blotches
	43 minutes	Brownish discoloration general.
	19½ hours	Many black blotches, dead-ripe appearance.
2.6	5 minutes	General color light brown, blotchy.
	17½ hours	Solid black, much moisture on surface.
3.2	½ minute	Brownish blotches general.
	8 minutes	Black blotches on brown, dead-ripe appearance.
	1½ hours	95 per cent of surface black, much moisture on surface.

A further observation included in the same paper was that the lenticels exhibit crimson-colored rings, which quickly become black. The depth of penetration was from three to five cells except under prolonged exposure. Exposure of five to seven days caused darkening one-half inch deep and a darkening of the vascular bundles. It was observed also that green pears are more seriously injured than ripe ones, and that increasing humidity increases the action of the ammonia.

In the present work no prolonged tests were made, the concentrations of ammonia used being high enough to show marked results in a very short time.

Bartlett pears that had not yet begun to become mellow were used. As shown in Table 4, changes in the color of the epidermis appeared in less than two minutes after they were subjected to ammonia. The injury appeared first around lenticels and abrasions, the ammonia apparently having no effect upon the color of the epidermal cells except those next to an opening. The coalescing of the spots, which occurs later, appears to be due to the diffusion of ammonia through the subepidermal tissues and not to any absorption through the cutinized, unbroken epidermis. It is also interesting to note that the pears remained reddish brown as long as they were exposed to the ammonia. When removed and held in the air at room temperature, the reddish brown color gradually turned darker and also spread until at least 50 per cent of the pear surface was black. (Pl. 1, B.) This change required about four hours. The pears exposed to the stronger ammonia were blotched brown and reddish brown when removed from the bell jar and became solid black within four hours. Penetration in the pears exposed to the less concentrated ammonia was confined to a depth of four or five cells below the epidermis; in the pears held in the more concentrated ammonia it reached greater depths. When fruit was exposed to the ammonia for 20 minutes, the vascular tissues were brown to a depth of 1.5 mm., and when held for a longer period the browning extended 8 to 10 mm. below the epidermis. These examinations were made 24 hours after the pears were removed from the ammonia.

Winter Nelis pears were exposed to ammonia (0.8 and 1.0 per cent) at a temperature of 70° F. and a relative humidity of 30 per cent. The rate of discoloration was slightly slower than that recorded for the higher percentages of ammonia (Table 4), but the results of these tests did not differ significantly from the others.

Anjou pears were subjected to ammonia fumes at low temperature. (Table 5.) The results indicate that lowering the temperature did not appreciably alter the rate of discoloration. Anjou pears are less susceptible than Winter Nelis.

BANANAS

The bananas used were light yellow with green ends and were quite firm. On exposure to ammonia, the color changes took place quickly in the weak ammonia, but progressed more rapidly in the more concentrated solution. (Table 4.) Even a short exposure caused a darkening of the peel, which persisted after removal from the ammonia chamber and gave the banana a dead-ripe appearance. (Pl. 1, C.)

Bananas were also exposed to ammonia fumes in the same way as Winter Nelis and Anjou pears. (Table 5.) The results were not sufficiently different to warrant more detailed discussion.

PEACHES

In addition to the changes in color (Table 4), peaches shriveled rapidly when removed from the ammonia. Three days after treatment they were dried and wrinkled and resembled mummies produced by brown rot. The flesh was brown throughout, indicating a more rapid and deeper penetration of the gas than was found in the other fruits studied.

DISCUSSION

As pointed out by Wheldale,[7] the reaction of the anthocyanins with alkalies and acids has been a matter of interest to investigators for many years. Although in this paper no attempt has been made to differentiate the various color pigments in the fruits and onions under consideration, it seems safe to assume that they are chiefly anthocyanins and anthoxanthins. The term anthocyanin is used here in a collective sense, to include the soluble pigments of blue, red, and violet tints of plants and plant products, while anthoxanthin[8] is used in a like manner to include the yellow and brown pigments. In his work on the relation of onion pigments to disease, Walker[9] states that the red and yellow pigments are solutes in the cell sap of the epidermal layer of the colored scales. He also mentions the fact that the pigmented cells of the yellowish varieties turn deep brownish yellow when treated with alkalies, a reaction typical of the flavones, and those of the red varieties turn pink in acid and green in alkaline solutions, a characteristic reaction of the anthocyanins. The anthocyanins are normally in solution in the cell sap, but sometimes the concentration becomes so great that the pigments separate out in a crystalline or an amorphous condition. This amorphous condition is generally found in the dry colored scales of cured onions that are placed upon the market. As the color-bearing scales die, their cell walls are frequently stained by exosmosis of the coloring matter, and it is along the broken edges of the scales where these cells are exposed that the first color reactions take place when onions are exposed to ammonia.

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[7] WHELDALE, M. THE ANTHOCYANIN PIGMENTS OF PLANTS. 318 p Cambridge. 1916.

With the possible exception of bananas, the surface discoloration of colored onions and fruits by ammonia is, therefore, to be attributed primarily to the action of the gas on the anthocyanins and anthoxanthins, while the end results are somewhat modified by the death of cells and by color changes incident to oxidation. The blackening of bananas occurs so soon after the initial discoloration that it is difficult to distinguish between the two reactions.

As indicated in the experiments described, ammonia injury may be merely a blemish, as in the discoloration of the outer dry color-bearing scales of the onion and the darkening of the lenticels of the apple and the pear, or it may be serious as in the softening and discoloring of the edible portions of onions and in the browning and softening of pears, bananas, and peaches. The strength of the ammonia fumes, the relative humidity, and the duration of the exposure determine the extent of the injury. Ordinarily, the injury produced by ammonia can be distinguished from other discolorations found in fruits and onions by the typical alkaline reaction produced with the pigments in the color-bearing surface tissues, although occasionally somewhat similar discolorations may be caused by other unfavorable conditions or by disease.

SUMMARY

Brownish and greenish black discolorations of onions and fruits that appear normal in every respect except color have frequently been observed on the Chicago market. Discolored onions have been found most often, but discolored apples, pears, peaches, and bananas also have been noted. The absence of a causal organism and the consistent association of the injury with storage indicated that the discoloration had resulted from exposure of the products to a uniformly distributed deleterious substance while held in storage.

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[8] WILLSTÄTTER, R., and EVEREST, A. E. UNTERSUCHUNGEN ÜBER DIE ANTHOCYANE. Liebigs Ann. Chem. 401: 189-232. 1913.

[9] WALKER, J. C. DISEASE RESISTANCE TO ONION SMUDGE. Jour. Agr. Research 24: 1019-1040, illus. 1923.

This type of injury was duplicated in the laboratory when onions and fruits were exposed to ammonia (0.8 to 29.3 per cent) at 70° F. and relative humidities of 30 and 85 per cent, and in cold storage at 31.5° with 0.8 to 3.2 per cent ammonia and a relative humidity of 83 per cent. The extent of the injury was determined by the percentage of ammonia in the air, the relative humidity, and the duration of the exposure. Variations in temperature did not greatly influence the rate or extent of discoloration.

It was demonstrated that the yellow, brown, and red pigment-bearing tissues become brownish to dark brown and in some cases greenish black when exposed to ammonia. The injury may be merely a blemish, as in the discoloration of the outer dry color-bearing scales of the onion and darkening of the lenticels of the apple and pear, or it may be serious, as in the softening and discoloring of onions and in the browning and softening of pears, bananas, and peaches.

THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF OPHIOBOLUS GRAMINIS IN PURE CULTURE[1]

By HURLEY FELLOWS

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INTRODUCTION

During the last few years Sewell and Melchers[2] have made some interesting observations on the destructiveness of take-all (*Ophiobolus graminis*) in the experimental wheat plots of the Kansas Agricultural Experiment Station at Manhattan, Kans. They noted that different kinds of cultivation in the plots apparently caused differences in the destructiveness of the take-all disease.

In inoculating wheat plants with *Ophiobolus graminis* the writer observed that sometimes, for no apparent reason, infection failed to develop. These inoculations were made and the inoculated plants kept in a greenhouse where the temperature was controlled and kept constantly favorable for infection. The soil moisture also was kept favorable for infection. Hence variation in the gas content of the soil suggested itself as a possible explanation of the apparent variations in the pathogenicity of the fungus.

Of the environmental factors that affect the development of a parasite in the soil, moisture, temperature, and acidity have received the most careful consideration from pathologists. Thus far little attention has been paid to the influence that changes in the gas content of the soil may exert on the growth and activity of the common soil-inhabiting parasites. As *Ophiobolus graminis* is influenced by different soil moistures and temperatures, which, in turn, affect the gas content of the soil, it was believed that, if the organism should prove sensitive to the variations in soil gases, its increase and activity might be controlled somewhat by any methods of cultivation that varied the gas content of the soil. With this in mind, the writer, in 1925, conducted a series of experiments in which pure cultures of *O. graminis* were grown in air containing different percentages of carbon dioxide and oxygen, respectively.

MATERIALS AND METHODS

The parasite was grown on solid media in Petri dishes and on liquid media in Erlenmeyer flasks. These were uniformly inoculated, each with a small piece of agar with new mycelium of the fungus, and placed in large glass jars closed with ground-glass tops. Through the top of

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[1] Received for publication May 22, 1928; issued November, 1928. These investigations were conducted in cooperation with the Kansas Agricultural Experiment Station, Manhattan, Kans., and this paper is No. 274 of the Department of Botany and Plant Pathology.

[2] SEWELL, M. C., and MELCHERS, L. E. THE EFFECT OF ROTATION AND TILLAGE ON FOOTROT OF WHEAT IN KANSAS, 1920–1924. Jour. Amer. Soc. Agron. 16: 768–771, illus. 1924.

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each jar three glass tubes were inserted and sealed in. One of these tubes extended to the bottom and served to admit the proper gas mixture that was to surround the cultures within. Another short tube permitted the removal of a small gas sample for analysis. The third tube had on its lower end a small rubber gas bag for keeping the barometric pressure inside the jar equal to that outside. The flask or Petri-dish cultures were placed in the bottom of the jar, which was then tightly sealed. A diagram of the apparatus is shown in Figure 1. The Petri-dish covers were raised slightly by means of small bent wires so placed that the gas in the Petri dishes could diffuse freely with that of the culture jar. The flasks were loosely plugged with cotton for the same reason.

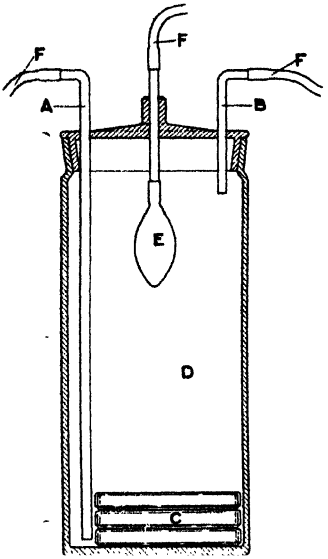

FIG. 1.—Diagram of a specimen jar used as a gas-control culture chamber. A, intake tube for gas mixtures; B, tube from which gas samples are taken for analysis; C, Petri dishes in which the organism is cultured; D, main portion of chamber in which various gas mixtures are retained; E, rubber gas bag used to keep the barometric pressure inside the jar equal to that on the outside; F, rubber-tube terminals upon which pinch cocks are placed

It was thought possible that the growth of the fungus might differ on different media, especially if one were solid and the other liquid, even though the concentrations of the gases of the surrounding atmosphere were constant. Accordingly, potato-dextrose agar and potato-dextrose decoction were selected as media. The growth measurements were made by weighing the dried mats of mycelium from the liquid medium and by measuring the diameters of the colonies on the agar medium.

Five different respective concentrations each of oxygen and carbon dioxide were used. (Tables 1 and 2.) When the concentration of carbon dioxide was varied that of oxygen was kept as near as possible to the percentage in the air, which by volume is approximately 21 per cent. However, when the oxygen was varied the concentration of carbon dioxide was 0.03 per cent or less. After the portions of oxygen or carbon dioxide necessary for a given percentage in a certain volume had been measured the remaining fraction of the volume was made up with nitrogen.

In the oxygen series, 22 to 24 agar cultures in Petri dishes were measured at each concentration, and 9 or 10 cultures from flasks were weighed. In the carbon-dioxide series 13 to 16 agar cultures in Petri dishes were measured at each concentration, and likewise for each concentration 3 or 4 flask cultures were weighed. It was not thought necessary to run more flask cultures in the carbon-dioxide series, as their responses were so similar to those of the agar cultures.

In these experiments where the oxygen or carbon dioxide was varied, the necessary gases were obtained from commercial pressure tanks. At the end of every 24-hour period the desired concentrations of oxygen and carbon dioxide, respectively, were made in a large graduated jar and then forced out of it into the culture jars by replacement with water. The measurements were easily made by replacing known quantities of water from the mixing jar with the gas to be measured. The exact percentages of carbon dioxide and oxygen in the culture jars were measured with a portable gas-analysis apparatus. At the beginning of the experiments the measurements were made immediately before and immediately after a new gas supply was placed in the culture jar. It was soon found, however, that a 24-hour period of growth did not materially alter the percentage of oxygen or carbon dioxide; therefore the measurements were made only immediately after the new supplies of gases were placed in the jars. In all cases the cultures were grown at room temperature, about 21° C., for seven days.

TABLE 1.—*Growth of Ophiobolus graminis at different concentrations of oxygen*

[Age 7 days; kept at about 21° C.]

Oxygen concentration	Colonies on potato-dextrose agar		Oxygen concentration	Mycelial mats on potato-dextrose decoction		
	Number measured	Average diameter		Number weighed	Weight	
					Total	Average
Per cent		*Cm.*	*Per cent*		*Gm.*	*Gm.*
0.8	23	1. 8	0. 2	([a])	([a])	([a])
6.2	23	5. 9	5. 2	10	0. 0912	0. 0091
10.8	23	6. 1	10. 0	9	. 3896	. 0433
13.9	24	4. 6	14. 8	10	. 6006	. 0601
21.3	22	5. 1	21. 2	10	. 6798	. 0680

[a] Growth slight or none.

TABLE 2.—*Growth of Ophiobolus graminis at different concentrations of carbon dioxide*

[Age 7 days; kept at about 21° C.]

Carbon dioxide concentration	Colonies on potato-dextrose agar		Carbon-dioxide concentration	Mycelial mats on potato-dextrose decoction		
	Number measured	Average diameter		Number weighed	Weight	
					Total	Average
Per cent		*Cm.*	*Per cent*		*Gm.*	*Gm.*
0.25	14	9. 04	0. 90	3	0. 3048	0. 1016
3.59	13	8. 62	3. 17	4	. 2630	. 0658
5.19	14	4. 61	5. 57	3	. 2696	. 0899
11.80	16	6. 69	11. 50	4	. 2276	. 0569
16.75	14	5. 50	18. 02	4	. 2120	. 0530

RESULTS

GROWTH OF THE FUNGUS AT DIFFERENT CONCENTRATIONS OF OXYGEN

The results from growing cultures on potato-dextrose agar in different percentages of oxygen are given in Table 1 and are shown graphically in Figure 2. At all concentrations of oyxgen used except the lowest (0.8 per cent) the organism showed growth at the end of seven days. At 6.2 per cent and 10.8 per cent growth was slightly better than that in the controls at 21.3 per cent, whereas at 13.9 per cent the growth was slightly less than that in the controls.

In potato-dextrose decoction the fungus usually grew slightly, even at a very low oxygen concentration. The small block of agar used to inoculate the solution usually showed fuzziness even in a trace of oxygen (0.2 per cent). In general, there was a gradual diminution in growth with the decrease in oxygen. This behavior of the fungus contrasted strikingly with its action on the solid medium, where with certain exceptions, growth was approximately equal to or better than the control until the oxygen was reduced below 6.2 per cent. (Table 1 and fig. 3.)

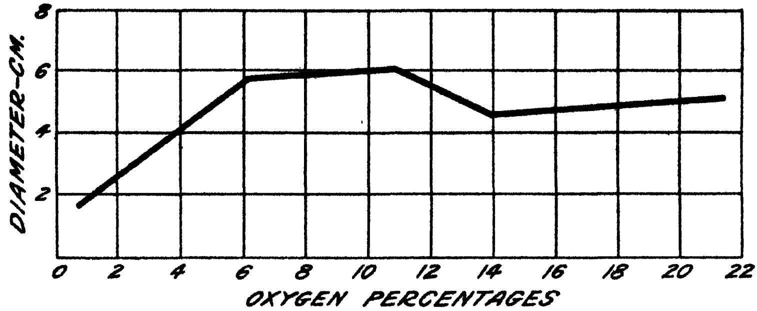

FIG. 2.—Growth of *Ophiobolus graminis* on potato-dextrose agar, at different percentages of oxygen. (Data in Table 1)

GROWTH OF THE FUNGUS AT DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE

On potato-dextrose agar the fungus grew fairly well at all concentrations of carbon dioxide. In general, increased quantities of carbon dioxide usually diminished growth somewhat, but this diminution was not regular. When graphed the averages of growth measurements show a bimodal curve; that is, there was good growth at certain concentrations of carbon dioxide and poorer growth at an intervening concentration. The growth at 5.19 per cent was only about half as great as at 3.59 per cent. At 11.8 per cent it increased again about one-half more than at 5.19 per cent. High concentrations of carbon dioxide seemingly do not seriously impair the growth of *Ophiobolus graminis* when it is cultured on potato-dextrose agar. The growth at 16.75 per cent carbon dioxide was

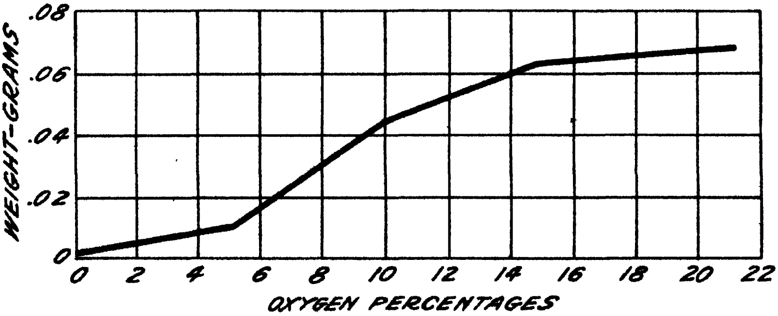

FIG. 3.—Growth of *Ophiobolus graminis* in potato-dextrose decoction, at different percentages of oxygen. (Data in Table 1)

about two-thirds of what it was at 0.25 per cent. (Table 2 and fig. 4.)

In general, the growth responses of the fungus in potato-dextrose decoction were similar to those on the solid medium. The curve of growth is bimodal and for the most part indicates a diminution of growth with an increase in carbon dioxide. The two best points of growth are at 0.9 and 5.57 per cent. It may be noted that the second high point of growth has shifted toward the lower concentration as compared to that on the solid medium. The higher concentrations of carbon dioxide do not decrease the rate of growth very greatly, for, at the highest concentration used, growth was no less than half as great as that at the lowest concentration. One should not expect as much diminution at high percentages of carbon dioxide as at low percentages of oxygen, for oxygen is demanded in metabolism, while carbon dioxide is a by-product. (Table 2 and fig. 5.)

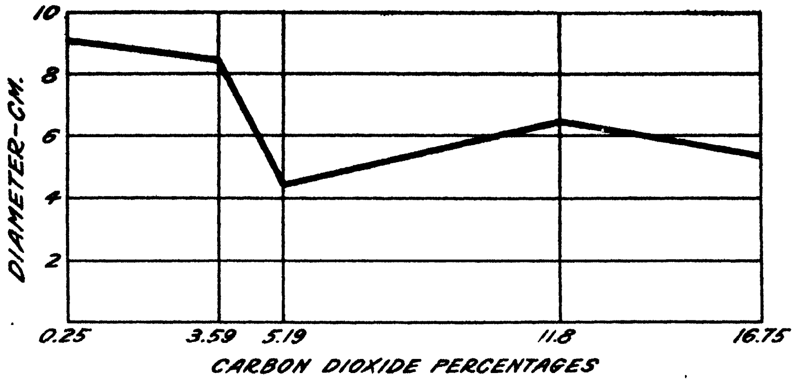

FIG. 4.—Growth of *Ophiobolus graminis* on potato-dextrose agar, at different percentages of carbon dioxide. (Data in Table 2)

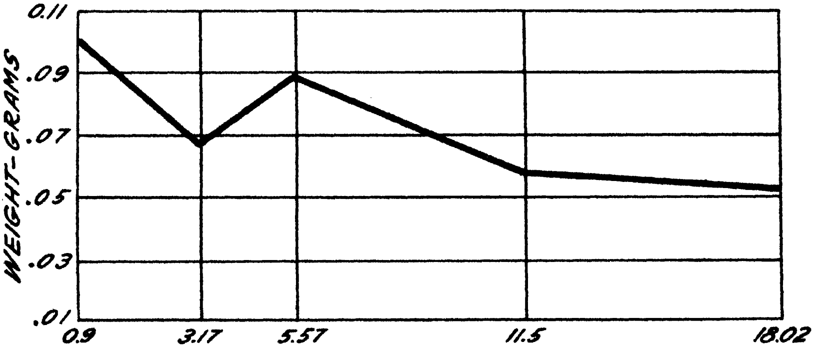

FIG. 5.—Growth of *Ophiobolus graminis* in potato-dextrose decoction, at different percentages of carbon dioxide. (Data in Table 2)

DISCUSSION

For a clearer understanding of the probable value of the experiments described in this paper it may be well to consider here the gaseous content of the soil. The two variable gases in the soil that mean most to the organisms that inhabit it are carbon dioxide and oxygen. There are two forms of these gases in all moist soil, namely, that dissolved in the soil water and that in the free gaseous state. It is a well-established fact that considerable quantities of carbon dioxide but only a trace of oxygen are dissolved in the soil water. This discussion is concerned with the gases found in cropped soils.

Two important pieces of work on soil gases are those of Russel and Appleyard [3] from Rothamsted, England, and of Leather [4] from Pusa, India. Russel and Appleyard worked chiefly, although not wholly, with the soil gases not in solution, while Leather included both the dissolved and the undissolved gases in his studies.

Russel and Appleyard found that the mean composition of the undissolved soil atmosphere on manured wheatland was as follows: Carbon dioxide in summer, 0.23 per cent, and in winter, 0.37 per cent; oxygen in summer, 20.74 per cent, and in winter, 20.31 per cent. On the same soil the greatest quantity of carbon dioxide ever found was 2.5 per cent in the early spring and the smallest quantity of oxygen about 18.4 per cent at the same time. In a water-logged soil the carbon dioxide was found to be as high as 9.1 per cent and the oxygen as low as 2.6 per cent. Such a condition, of course, is exceptional.

In the analyses reported by Leather both the dissolved and the undissolved gases were included. One of his representative samples was from a field growing corn (*Zea mays*). The maximum carbon dioxide content found at any time was 12.3 per cent and the minimum oxygen 6.28 per cent. The maximum quantity of carbon dioxide ever found at any time in any field used for crops was 18.4 per cent. At the same time the oxygen content was 10.6 per cent. These later analyses were made from three to nine days after a green-manure crop had been plowed under. Leather's analyses of the soil solution alone, taken when the soil was in good condition for holding oxygen, showed that, by volume, the soil solution held only 0.00607 per cent oxygen.

Ophiobolus graminis is a surface grower. If the inoculum is placed below the surface of a liquid medium the hyphae come to the surface before any extensive growth occurs. If the inoculum is placed at the surface the penetration into the liquid below is very slight. Therefore it may be assumed that *O. graminis* is more dependent for its growth and activity on the undissolved soil atmosphere than on the dissolved gases in the medium below.

From the laws of gaseous behavior, it would appear that the two soil atmospheres are not abruptly parted at the surface of water films, but that at this point there is more or less equilibrium between the two. This fact increases the difficulty of determining the percentages of the various gases to which the organism is exposed below the surface of its medium. Since *Ophiobolus graminis* is a surface grower, it is highly probable that the undissolved gases contribute most to its development. The small quantity of oxygen in the soil liquids, as shown by Leather's analyses, also indicates that the oxygen in the undissolved soil atmosphere is the more important. Laboratory technic has not yet been developed to the point where it is possible to determine the difference in the gas content of the surface and the deeper layers of thin aqueous films. Accordingly, one can not tell exactly what percentages of gases are in that portion of the medium in which the organism is growing.

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[3] RUSSEL, E. J., and APPLEYARD, A. THE ATMOSPHERE OF THE SOIL: ITS COMPOSITION AND THE CAUSES OF VARIATION. Jour. Agr. Sci. [England] 7: 1-48, illus. 1915.

[4] LEATHER, J. W. SOIL GASES. India Dept. Agr. Mem., Chem. Ser. 4: 85-134, illus. 1915.

As far as these experiments have shown, the gases ordinarily found in soils, whether free or in solution, are not present in sufficient quantity to affect the growth of *Ophiobolus graminis* very materially. Even the extreme quantities found by Russel and Appleyard on manured wheatlands in spring would not appreciably modify growth. The soil on which wheat is ordinarily grown in the wheat regions of the United States is seldom given organic fertilizers, and the carbon dioxide content therefore should rarely be as high or the oxygen as low as on the Rothamsted plots.

This paper presents but one phase of a pathological problem. To complete the study, wheat plants must be inoculated with *Ophiobolus graminis* and then grown in various concentrations of oxygen and carbon dioxide, respectively. Plans are under way to do this.

SUMMARY

Variations in the destructiveness of take-all in the field and irregularities in some greenhouse experiments suggested a series of tests to determine what effect different quantities of carbon dioxide and oxygen in the surrounding atmosphere might have on the growth of *Ophiobolus graminis*, the fungus causing take-all of wheat.

The cultures used were grown on potato-dextrose agar and potato-dextrose decoction. The two media were selected in order that the growth of the fungus might be observed on liquid and solid media when atmospheric conditions were the same.

On both the liquid and the solid media the organism grew in all the oxygen concentrations used. In the liquid medium, growth diminished gradually as the oxygen concentration decreased; on the solid medium, marked diminution did not occur until the oxygen was below 6 per cent. A very small percentage of oxygen greatly reduced growth.

The fungus grew well on both liquid and solid media when the carbon dioxide content was varied, although at the highest carbon-dioxide concentration used, 18.02 per cent, some diminution in growth occurred. In both of the carbon dioxide series; that is, the liquid-medium series and the solid-medium series, the growth curve was distinctly bimodal.

It is believed that the variations in carbon dioxide and oxygen as found in arable soils are not great enough to affect materially the growth of *Ophiobolus graminis*.

DAILY GROWTH AND OIL CONTENT OF FLAXSEEDS[1]

By A. C. Dillman

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INTRODUCTION

The yield of oil in flaxseed is of first importance to the linseed crusher because it is a large factor in the cost of oil obtained from different lots of seed. Mature flaxseed contains from 35 to 45 per cent of oil. It is important to know whether immature seed contains the same proportion of linseed oil as is found in the fully ripened seed.

Immature seed results from harvesting flax before it is fully ripened,— as is sometimes necessary with late-sown flax in order to avoid possible damage from frost. It is the practice also to harvest fiber flax while the stems are still green and before the seed is fully ripe, in order to obtain a better quality of fiber. Such immature seed usually brings a lower price on the market, although there is very little definite information in regard to the oil content of immature seed. An extensive series of analyses recently completed by Coleman and Fellows (*2*)[3] indicates, however, that immature, frosted, or weather-damaged flaxseed may contain as much linseed oil (on basis of dry weight) as seed normally matured.

In order to determine at what stage of growth the oil is laid down in the developing flaxseed, the writer undertook a study of the daily growth of the seed and of the inclosing boll or capsule. This included daily measurements of the seeds from the day of flowering to full maturity, the wet and dry weights, and the oil determination of samples at regular intervals. An attempt was made, also, to determine by microchemical tests what changes occur in the early development of the seed.

The flax plant is convenient to work with in a study of this kind. The flowers normally are self-fertilized, so that uniform plants of pure lines may be obtained easily. The flowers open and pollination occurs in the early morning, and flowering of the plant continues for a period of a week or 10 days. With some varieties, especially in cool, wet weather, flowering may continue for a much longer period, so that sometimes ripe bolls and flowers may be found on the same plant. (Fig. 1.) This fact is referred to in connection with the

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[1] Received for publication July 13, 1928; issued November. 1928.

[2] The writer wishes to express his thanks to Dr. C. O. Appleman, of the University of Maryland, for assistance in outlining these experiments, and to A. C. Arny, agronomist, University Farm, St. Paul, Minn., for helpful suggestions and for the use of materials and laboratory equipment. The writer appreciates, also, the help of Frank Stevenson, who assisted in the work at University Farm, J. C. Brinsmade, jr., assistant agronomist, Office of Cereal Crops and Diseases, who obtained the material for the oil determinations at Mandan, N. Dak., and of D. A. Coleman and H. C. Fellows, Grain Division, Bureau of Agricultural Economics, for use of equipment and for assistance in making the oil determinations. The data obtained in 1926 were included in a thesis submitted to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the degree of master of science, June, 1926.

[3] Reference is made by number (italic) to "Literature cited," p. 377.

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review of "Earlier investigations" herein reported. The flax boll contains 10 seeds, when all develop, and the seeds are removed easily for measurement, weighing, and analysis.

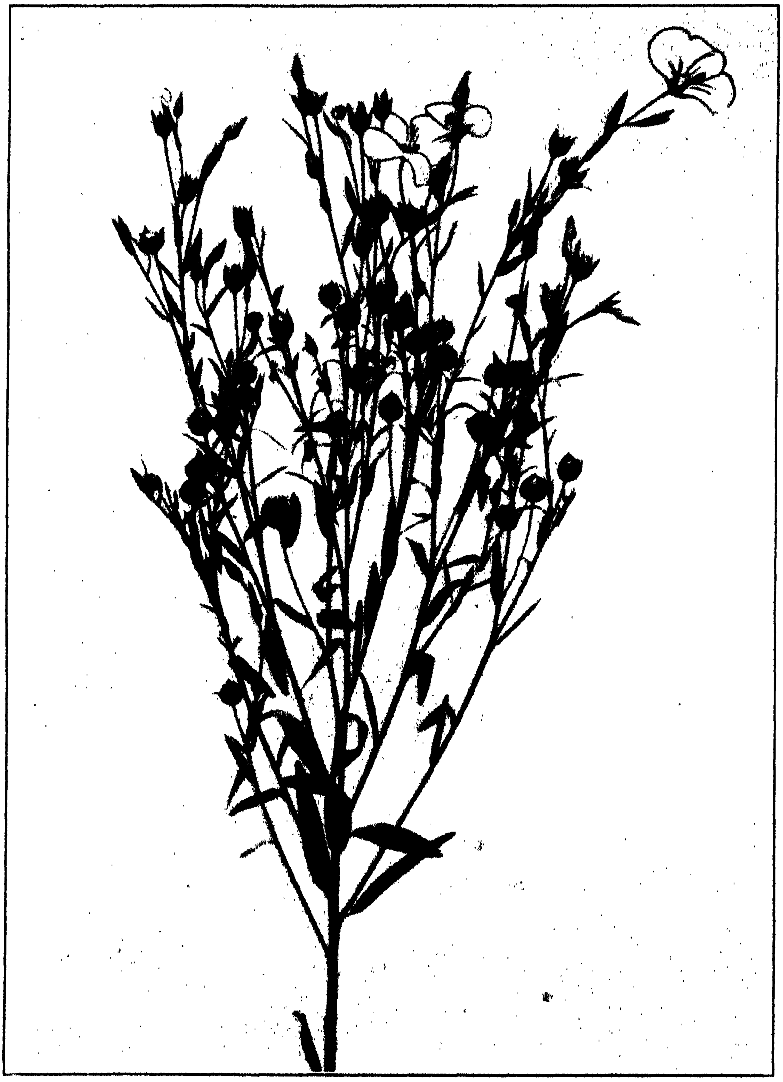

FIG. 1.—A panicle of flax, showing buds and flowers and bolls in successive stages of growth, some of the latter being fully mature

EARLIER INVESTIGATIONS

Few investigations have been made of the deposit of oil in the growing flaxseed. Most of the available studies apparently were made with all of the seed from plants harvested at different stages of growth. As previously mentioned, the flax plant blooms for a period of one or two weeks, a few flowers appearing each day, so that bolls in successive stages of development are found on the same plant. Samples from an entire plant will contain seeds of different stages of development, depending upon the age of the individual capsules. The relative proportion of seeds of the different ages found in the samples will determine, therefore, the percentage of oil found by analyses.

Ivanow (*5*) in 1907 and 1910 determined the percentage of oil in flaxseed from plants harvested at four different stages of growth. In 1907 he obtained results ranging from 33.8 per cent of oil in samples described as "seeds green with much sap" to 41.9 per cent in "seeds fully ripe." In 1910 he obtained a much wider range in oil content. From plants harvested on July 5, about one week after flowering, the immature seeds contained 4.4 per cent of oil. From plants harvested on July 18, having "seeds unripe, sappy, green," the seeds contained 11 per cent of oil. Plants harvested on August 3, with "seeds unripe," yielded 32.5 per cent of oil, while plants harvested on August 25 with "seeds fully ripe" yielded 35 per cent of oil.

Eyre and Fisher (*3*), in a study of linseed in England in 1914, obtained a range from 21 per cent to 40.9 per cent of oil. They collected plants having green and ripe bolls and separated the seeds into four groups arranged in order of increasing degrees of ripeness and found the oil content to be as follows:

Seed quite green, 21.05 per cent.
Seed just beginning to turn brown, 30.08 per cent.
Seed wholly brown but not loose in capsule, 38.03 per cent.
Seed fully ripe, i. e., quite loose in capsule, 40.88 per cent.

Washburn (*6*), at the North Dakota Agricultural Experiment Station, found only a slight difference in the percentage of linseed oil in plants harvested August 20 when the seeds were "black to green and very light in weight" and plants harvested as late as September 26 when fully mature. The extreme range was from 38.8 to 40.8 per cent.

Coleman and Fellows (*2*) separated and analyzed immature seeds in comparison with mature sound seeds from the same bulk samples. The eight samples of immature seeds averaged 32.62 per cent of oil as compared with 41.35 per cent in the mature seeds. The unripe seeds represented only a very small proportion of the sample.

Robinson[4] working at East Lansing, Mich., in 1926, studied the oil content of seeds of a variety of fiber flax, Saginaw, during a period of 30 days, beginning on July 7 when the plants were still in bloom. The plants were pulled on successive days and dried in the open air under cover before the seeds were removed for analysis. The oil content of the seeds ranged from 22.9 per cent from plants gathered July 7 to 34.5 per cent from mature plants gathered August 5.

Bushey, Puhr, and Hume (*1*), at the South Dakota Agricultural Experiment Station, determined the oil content of flaxseed collected at five dates in 1926. The samples were taken from a plot seeded rather late, June 10. The variety is not mentioned. The oil content ranged from 29.51 per cent on August 25 when "about one-third of the seeds were greenish in color and some flowers were still present," to 36.84 per cent on September 21 when the plants were fully mature. On October 1 when the plants were "dead ripe" the seeds contained 37.01 per cent of oil.

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[4] The unpublished data from these experiments were kindly furnished the writer by B. B. Robinson, of the Office of Fiber Plants, Bureau of Plant Industry, U. S. Department of Agriculture. The experiments were carried on in cooperation with the Michigan Agricultural Experiment Station.

PRESENT INVESTIGATIONS

The investigations reported in this paper consist of two principal studies: (1) The daily growth in volume of the flaxseed and (2) the laying down of oil in the developing seed. Measurements of the daily growth of the seed were made only at University Farm, St. Paul, Minn., in 1926. The daily wet weight, dry weight, and percentage of moisture of 100 seeds were obtained at two-day and three-day intervals at St. Paul in both 1926 and 1927. The oil content of flaxseed, collected at regular intervals after flowering, was determined at St. Paul, Minn., and Mandan, N. Dak., during both years. A comparison of the rate of oil formation of seed formed during the period of early flowering (flowers tagged July 2) and seed formed during the period of late flowering (flowers tagged July 12) was made at St. Paul in 1927.

EXPERIMENTAL METHODS

Seeds of uniform and known age were obtained by tagging flax flowers on the day of blooming. This method was used by Harlan (*4*) in a study of the daily growth of the barley kernel.

The variety of flax studied was Rio (C. I. 280), a selection (L. 79) made in 1918 by H. D. Long, of the North Dakota Agricultural Experiment Station, from a sample of imported Argentine seed. This variety was chosen because it has large seeds, is uniform in type, and can be easily distinguished from other varieties, so that one can be sure of working with a uniform and pure line. The plants are short to midhigh (14 to 24 inches), with usually two or more basal branches; the flowers are large, 18 to 24 mm. wide; petals blue with darker veins; anthers blue; filaments white; style white or with a trace of light blue at the base; the bolls are large (7 to 7.5 mm. in diameter) and slow to dehisce; the false septa between the seeds are ciliate on the margin; seeds large (weight about 8 gm. per 1,000 seeds). This variety is highly resistant to wilt and immune to rust, but it is susceptible to pasmo. It often produces high yields of seed, and the seed has a high oil content. The variety, however, does not appear to endure drought or high temperature during the blossoming period, such conditions causing many flowers to blight.

Plants used for the study were growing in the border rows of plots of the chosen variety in the varietal experiments. These plots were 132 feet long and separated from adjoining plots by alleys 30 inches wide. (Fig. 2.) When the plants were from 10 to 15 inches high and in the period of early blooming, enough flowers were tagged to supply seeds for the daily measurements of growth and for oil determinations at regular intervals. The flowers were marked by tying a bit of colored woolen yarn around the flower stalk by a single loose knot, all flowers tagged on one day being marked with one color of yarn. Flowers were tagged at St. Paul in 1926 as follows:

June 18.—550 flowers, red yarn.
June 19.—600 flowers, yellow yarn.
June 22.—750 flowers, pink yarn.
June 24.—820 flowers, white yarn.

For the growth studies 15 or 18 bolls were collected each morning at 8 o'clock and taken to the laboratory as material for measurement and weight determinations. The bolls were kept in a glass jar until used, with a pad of moist cotton to prevent drying.

The lengths and diameters of 10 or more bolls were measured with a small vernier caliper, reading to 0.1 mm. The length, width, and thickness of the seeds were determined by measuring 5 seeds from each of 5 bolls, a total of 25 seeds each day. The measurements were made with a dissecting binocular fitted with a micrometer by which readings could be made directly to 0.1 mm. The 5 seeds from each boll were placed on a glass slide and the length and width recorded in succession. The seeds were then turned on edge, one at a time, with a needle, and the thickness recorded.

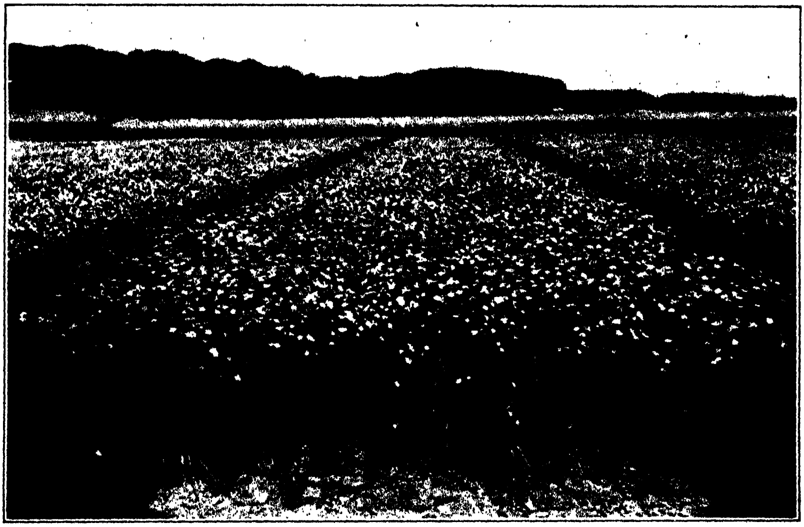

FIG. 2.—Plot of Rio flax (C. I. 280) at University Farm, St. Paul, Minn., in full bloom, June 30, 1926

DAILY GROWTH IN VOLUME

The average daily growth in volume, as shown by the increased length and diameter of the boll and by the length, width, and thickness of the seed, is shown in Table 1, and graphically in Figure 3. The first measurements here recorded were made on June 18, when the boll (ovary) and ovules of 10 flowers were measured. After this date measurements of 10 bolls and of 25 seeds were made daily.

The period of growth extended from June 18, when the flowers were tagged, to July 26. The first part of the period included the season having the most hours of daily sunshine, about 15½ hours per day in the latitude of St. Paul.

The growth in volume of the boll and of the seed, as determined by the measurements, was surprisingly rapid and uniform up to the thirteenth day, when about the maximum size was attained. There appears to have been some decrease in volume from the fifteenth to the seventeenth days, inclusive, but this probably is not significant. Apparently there was very little change in the size of the bolls or of the seeds, as determined by the dimensions, from the thirteenth day after flowering to full maturity.

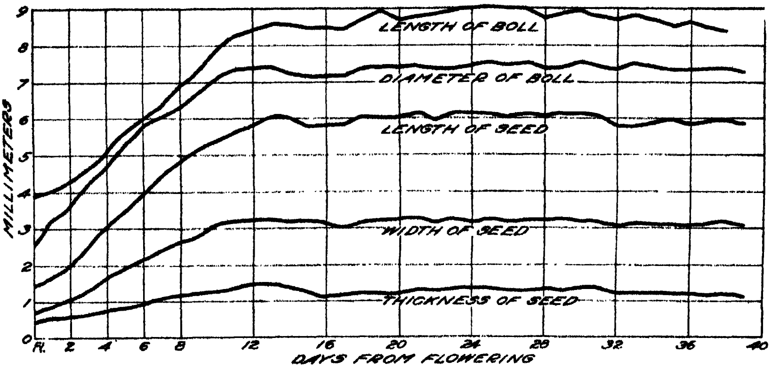

FIG. 3.—Average daily length and diameter of bolls of Rio flax (C. I. 280) and length, width, and thickness of seeds, from flowering to maturity, at University Farm, St. Paul, Minn., in 1926

TABLE 1.—*Average measurements of 10 bolls and of 25 seeds of Rio flax on successive days (except as noted) from flowering on June 18 to maturity, at University Farm, St. Paul, Minn., in 1926*

Time from flowering	Date of sampling	Measurements				
		Bolls		Seeds		
		Length	Diameter	Length	Width	Thickness
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
In flower	June 18	3.78	2.67	1.46	0.71	0.48
1 day	June 19	4.08	3.22	1.71	.90	.58
2 days	June 20	4.32	3.64	1.98	1.06	.60
3 days	June 21	4.70	4.31	2.48	1.36	.68
4 days	June 22	5.09	4.85	3.14	1.71	.80
5 days	June 23	5.72	5.33	3.53	1.93	.85
6 days	June 24	6.05	5.89	4.02	2.20	1.00
7 days	June 25	6.38	6.00	4.52	2.42	1.11
8 days	June 26	6.90	6.34	4.88	2.62	1.18
9 days	June 27	7.27	6.75	5.19	2.81	1.24
10 days	June 28	7.96	7.18	5.44	3.14	1.33
11 days	June 29	8.32	7.35	5.65	3.19	1.41
12 days	June 30	8.41	7.31	5.84	3.24	1.49
13 days	July 1	8.70	7.46	6.11	3.29	1.49
14 days	July 2	8.72	7.39	6.04	3.34	1.44
15 days [a]	July 7	8.44	7.14	5.80	3.23	1.23
16 days [a]	July 8	8.56	7.20	5.78	3.13	1.18
17 days [a]	July 9	8.45	7.20	5.85	3.05	1.24
18 days	July 6	8.79	7.42	6.05	3.23	1.22
19 days	July 7	8.98	7.45	6.09	3.26	1.26
20 days	July 8	8.72	7.45	6.08	3.30	1.24
21 days	July 9	8.89	7.51	6.20	3.32	1.36
22 days	July 10	8.86	7.38	6.05	3.20	1.29
23 days	July 11	8.98	7.37	6.18	3.30	1.38
24 days	July 12	9.09	7.50	6.19	3.19	1.36
25 days	July 13	9.14	7.61	6.20	3.31	1.37
26 days	July 14	9.00	7.54	6.09	3.26	1.36
27 days	July 15	9.10	7.63	6.20	3.30	1.32
28 days	July 16	8.76	7.35	6.10	3.26	1.37
29 days	July 17	8.94	7.44	6.18	3.32	1.35
30 days	July 18	8.99	7.56	6.17	3.23	1.37
31 days	July 19	8.92	7.45	6.16	3.24	1.40
32 days	July 20	8.83	7.44	5.84	3.17	1.27
33 days	July 21	8.93	7.57	5.82	3.19	1.30
34 days	July 22	8.76	7.47	5.97	3.14	1.30
35 days	July 23	8.56	7.36	6.00	3.16	1.25
36 days	July 24	8.72	7.37	5.90	3.12	1.28
37 days	July 25	8.51	7.44	6.02	3.18	1.19
38 days	July 26	8.46	7.46	6.01	3.23	1.26
Bulk ripe	Aug. 2	8.84	7.33	5.93	3.08	1.16

[a] Tagged June 22.

There was more or less variation in the size of the different bolls collected each day. This will be seen by reference to Table 2, where the actual measurements of the individual bolls and seeds are given for the first, sixth, twelfth, eighteenth, twenty-fourth, and thirtieth days of growth. As would be expected, there was an evident correlation between the size of the individual boll and of the seeds contained in it. The degree of correlation has not been determined. The data in Table 2 have been presented to indicate the range of variation encountered. Similar data were obtained for each day of the growth period, but it is considered unnecessary to present all of the detailed data in this paper.

TABLE 2.—*Measurements of individual bolls and seeds of Rio flax at definite intervals after flowering, at St. Paul, Minn., in 1926*

Sample	Measurements					Sample	Measurements				
	Bolls		Seeds				Bolls		Seeds		
	Length	Diameter	Length	Width	Thickness		Length	Diameter	Length	Width	Thickness
	June 19, 1 day after flowering						June 24, 6 days after flowering				
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
No 1	4.3	3.2	1.7 1.7 1.6 1.7 1.7	0.9 .8 .8 .9 .9	0.6 .6 .7 .6 .5	No. 1	5.6	5.5	3.9 3.9 3.9 3.9 3.9	1.9 2.0 2.0 2.0 2.0	0.9 1.0 1.0 1.0 1.0
No. 2	3.7	3.0	1.6 1.6 1.6 1.6 1.6	.7 .8 .8 .9 .8	.6 .6 .6 .6 .6	No. 2	6.0	5.8	4.1 4.1 4.1 4.2 4.1	2.2 2.2 2.2 2.2 2.2	1.0 1.0 1.0 1.0 1.0
No. 3	4.0	3.2	1.7 1.7 1.7 1.7 1.7	.9 .8 .9 .9 .9	.6 .5 .5 .6 .6	No. 3	6.1	6.0	4.3 4.3 4.4 4.3 4.3	2.3 2.3 2.3 2.4 2.4	1.0 1.0 1.0 1.0 1.0
No. 4	4.0	3.2	1.6 1.7 1.7 1.6 1.7	.9 .9 .9 .8 .9	.5 .6 .6 .5 .6	No. 4	6.2	6.0	4.2 4.2 4.3 4.2 4.3	2.2 2.3 2.3 2.3 2.2	1.0 1.0 1.0 1.0 1.0
No. 5	4.5	3.5	1.9 1.9 1.9 2.0 1.9	1.0 1.1 1.0 1.1 1.0	.6 .6 .6 .6 .6	No. 5	6.3	6.0	4.2 4.2 4.3 4.2 4.3	2.2 2.1 2.3 2.2 2.2	1.0 1.0 1.0 1.0 1.0
No. 6	4.1	3.2				No. 6	6.0	5.8			
No. 7	3.9	3.1				No. 7	6.1	5.9			
No. 8	4.4	3.3				No. 8	6.0	5.9			
No. 9	4.2	3.3				No. 9	6.1	6.0			
No. 10	3.8	3.2				No. 10	6.1	6.0			
Average	4.00	3.22	1.71	.89	.58	Average	6.05	5.89	4.16	2.20	1.00

TABLE 2.—*Measurements of individual bolls and seeds of Rio flax at definite intervals after flowering, at St. Paul, Minn., in 1926*—Continued

ample	Measurements					Sample	Measurements				
	Bolls		Seeds				Bolls		Seeds		
	Length	Diameter	Length	Width	Thickness		Length	Diameter	Length	Width	Thickness
	June 30, 12 days after flowering						July 12, 24 days after flowering				
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
No. 1	8.4	7.2	5.8 5.7 6.0 6.0 5.8	3.0 3.1 3.2 3.1 3.4	1.8 1.5 1.4 1.5 1.5	No. 1	9.5	7.3	6.3 6.3 6.1 6.2 6.2	3.0 3.2 3.2 3.2 3.2	1.3 1.2 1.4 1.2 1.2
No. 2	8.3	7.3	5.6 5.8 5.6 5.6 6.0	3.2 3.3 3.1 3.4 3.2	1.5 1.5 1.3 1.5 1.5	No. 2	9.0	7.3	6.3 6.3 6.1 6.1 6.4	3.2 3.2 3.1 3.2 3.3	1.5 1.3 1.4 1.5 1.4
No. 3	8.0	7.3	6.0 6.0 6.0 5.7 5.9	3.3 3.3 3.4 3.2 3.2	1.5 1.5 1.5 1.4 1.6	No. 3	9.0	7.4	6.2 6.2 6.2 6.0 6.0	3.3 3.3 3.2 3.0 3.3	1.3 1.4 1.4 1.4 1.3
No. 4	8.3	7.2	5.6 5.7 6.0 5.6 5.8	3.3 3.1 3.3 3.3 3.2	1.4 1.4 1.5 1.5 1.5	No. 4	9.3	7.1	6.2 6.2 6.1 6.3 6.2	3.1 3.1 3.1 3.2 3.2	1.3 1.5 1.3 1.3 1.3
No. 5	8.6	7.4	6.0 6.0 5.9 5.9 6.0	3.2 3.3 3.4 3.1 3.3	1.5 1.5 1.4 1.5 1.5	No. 5	8.8	7.6	6.1 6.2 6.2 6.2 6.2	3.1 3.3 3.4 3.3 3.3	1.4 1.4 1.4 1.4 1.3
No. 6	8.3	7.5				No. 6	9.3	7.7			
No. 7	8.7	7.4				No. 7	9.1	7.8			
No. 8	8.1	7.0				No. 8	9.0	7.6			
No. 9	8.7	7.6				No. 9	9.0	7.8			
No. 10	8.7	7.4				No. 10	8.9	7.4			
Average	8.41	7.33	5.84	3.24	1.49	Average	9.09	7.50	6.19	3.20	1.35
	July 6, 18 days after flowering						July 18, 30 days after flowering				
No. 1	8.6	7.3	6.0 5.9 5.9 5.9 5.9	3.2 3.2 3.3 3.3 3.3	1.2 1.2 1.2 1.2 1.2	No. 1	8.8	7.5	5.9 5.9 5.9 5.9 5.9	3.2 3.1 3.1 3.1 3.2	1.2 1.3 1.3 1.2 1.3
No. 2	9.0	7.5	6.0 6.2 6.0 6.2 6.1	3.3 3.3 3.1 3.0 3.2	1.3 1.2 1.2 1.4 1.2	No. 2	9.1	7.6	6.2 6.2 6.2 6.2 6.1	3.3 3.3 3.3 3.3 3.1	1.5 1.4 1.3 1.4 1.4
No. 3	9.1	7.5	6.1 6.1 6.1 6.2 6.3	3.4 3.4 3.3 3.4 3.3	1.2 1.2 1.2 1.2 1.3	No. 3	8.7	7.3	6.1 6.1 6.0 6.1 6.2	3.3 3.1 3.0 3.1 3.1	1.3 1.3 1.5 1.4 1.5
No. 4	8.9	7.3	5.9 6.0 6.0 6.0 5.9	3.0 3.1 3.3 3.2 3.1	1.2 1.1 1.1 1.2 1.3	No. 4	8.1	7.5	6.3 6.4 6.3 6.3 6.3	3.3 3.2 3.3 3.2 3.3	1.4 1.5 1.5 1.4 1.4
No. 5	9.0	7.3	6.2 6.1 6.1 6.0 6.1	3.2 3.2 3.2 3.2 3.2	1.2 1.2 1.2 1.3 1.2	No. 5	9.0	7.6	6.3 6.3 6.3 6.4 6.4	3.3 3.3 3.4 3.4 3.4	1.4 1.4 1.3 1.4 1.3
No. 6	8.9	7.5				No. 6	9.3	7.5			
No. 7	9.1	8.0				No. 7	9.0	7.6			
No. 8	8.0	7.6				No. 8	9.3	7.6			
No. 9	8.8	7.8				No. 9	9.3	7.8			
No. 10	8.8	7.6				No. 10	9.3	7.6			
Average	8.82	7.54	6.05	3.23	1.22	Average	8.99	7.56	6.17	3.23	1.37

DAILY GROWTH IN WEIGHT

The wet weight, dry weight, and percentage of moisture in flaxseed, collected each day from flowering to maturity at St. Paul in 1926, are shown in Table 3. In 1927 the samples were taken on the fifth, seventh, and ninth days after flowering and at three-day intervals thereafter to maturity. These data also are shown in Table 3. A comparison of the rate of growth, as measured by the increase in wet and dry weights, is represented graphically in Figure 4.

The curves for the daily increase in wet weight are similar to the growth curves shown in Figure 3, the wet weight reaching nearly its maximum at 12 days after flowering. After the twelfth day the wet weight increased only slightly up to the thirty-sixth day, after which it declined rapidly during the period of ripening. The curves for 1926 and 1927 agree very closely.

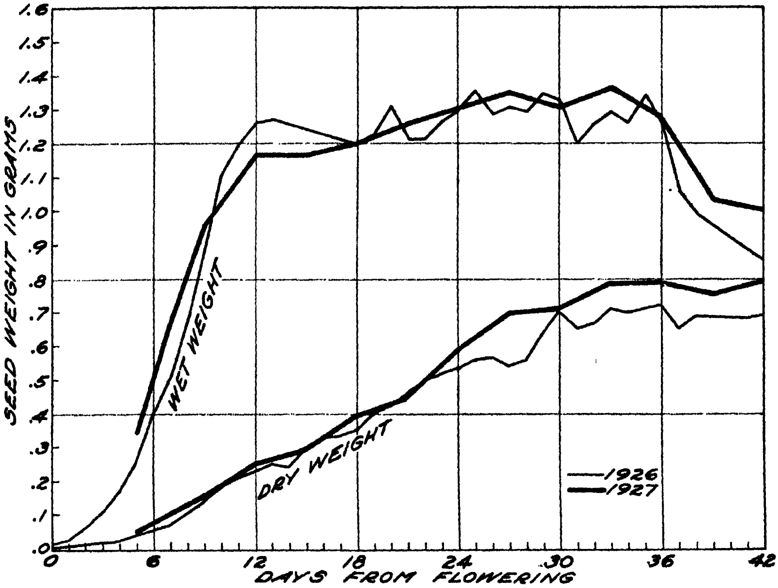

FIG. 4.—Wet and dry weights of 100 seeds of Rio flax, grown at University Farm, St. Paul, Minn., determined daily in 1926 and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927

The dry weight increased slowly during the first four days. From that time there was a more rapid and fairly uniform daily increase in dry weight up to the thirty-sixth day, when the maximum weight was attained. This point probably represents the stage of maximum growth and full maturity except for the loss of moisture in ripening. The dry weight remained constant during the process of ripening when, as noted above, the wet weight declined rapidly.

Figure 5 shows the percentage of moisture present in the seeds (based on the wet weight) from flowering to maturity. The percentage of moisture continued nearly uniform at about 85 per cent up to the eighth or ninth day. From the tenth to the thirty-fifth days there was a more or less steady decrease coincident with the increase in dry weight. The rapid decrease in the percentage of moisture after the thirty-sixth day conforms with the decrease in wet weight during the ripening stage.

TABLE 3.—*Daily wet and dry weights of 100 seeds of Rio flax at University Farm, St. Paul, Minn., showing the percentage of moisture from day of flowering to maturity in 1926 and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927*

Time from flowering	1926			1927		
	Wet weight	Dry weight	Moisture	Wet weight	Dry weight	Moisture
	Grams	*Gram*	*Per cent*	*Grams*	*Gram*	*Per cent*
In flower	0.0212	0.0032	85			
1 day	.0288	.0040	86			
2 days	.0600	.0098	84			
3 days	.1054	.0172	84			
4 days	.1606	.0236	86			
5 days	.2612	.0380	85	0.3378	0.0500	85
6 days	.4248	.0624	85			
7 days	.5180	.0768	85	.6735	.1042	85
8 days	.6920	.1046	85			
9 days	.8788	.1434	84	.9640	.1570	84
10 days	1.1174	.1852	83			
11 days	1.2016	.2140	82			
12 days	1.2588	.2298	82	1.1708	.2470	79
13 days	1.2732	.2500	80			
14 days	1.1112	.2440	78			
15 days	1.0820	.2928	73	1.1744	.2890	75
16 days	1.1530	.3310	71			
17 days	1.1012	.3326	70			
18 days	1.2040	.3540	71	1.2031	.3832	68
19 days	1.2252	.3930	68			
20 days	1.3076	.4200	68			
21 days	1.2206	.4646	62	1.2594	.4456	65
22 days	1.2208	.4878	60			
23 days	1.2730	.5178	59			
24 days	1.2956	.5276	59	1.3122	.5824	56
25 days	1.3604	.5576	59			
26 days	1.2876	.5600	57			
27 days	1.3174	.5428	59	1.3472	.6914	49
28 days	1.3022	.5516	58			
29 days	1.3482	.6426	52			
30 days	1.3388	.7008	48	1.3086	.7090	46
31 days	1.2056	.6514	46			
32 days	1.2680	.6764	47			
33 days	1.2942	.7102	45	1.3721	.7850	43
34 days	1.2712	.6992	45			
35 days	1.3231	.7115	46			
36 days	1.2498	.7218	42	1.2750	.7874	38
37 days	1.0768	.6532	39			
38 days	.9932	.6808	31			
39 days	.8880	.6716	24	1.1316	.7602	33
40 days	.8740	.6820	22			
42 days				1.0280	.7970	22

PERIOD OF OIL FORMATION

Perhaps the most important phase of the investigation was that concerning the time at which the oil is laid down in the developing seed. This was studied at St. Paul and at Mandan in both 1926 and 1927. In 1926 the determinations were made on samples taken at intervals of three days, beginning on the ninth day after flowering. One sample, however, was collected on the eighth day at St. Paul. In 1927, samples of seed were obtained on the fifth, seventh, and ninth days after flowering and thereafter at three-day intervals to full maturity. (Fig. 6.)

Different methods of drying the seeds before making the oil determinations were employed at St. Paul and at Mandan, but it is believed that accurate results were obtained by both methods. At Mandan the green bolls were placed in thin gauze bags and hung in a well-ventilated attic to dry. As the weather was clear and warm, the drying was rapid, and it is probable that very little change in the oil content of the seeds occurred after the bolls were gathered. At St. Paul it was the original plan to heat the green bolls in a closed Petri dish in order to stop growth and enzyme action in the seeds, then dry the bolls in an oven, and finally remove the seeds for analysis. The heating, unfortunately, shrunk the very immature bolls so that the seeds could not be separated.

The method finally adopted was to remove the seeds from the fresh bolls. A thin section was cut from the base of the boll, exposing the ends of the seeds. Then, by gently rolling the boll between the fingers, the seeds were pressed out. As the bolls became more woody, after 18 or 20 days of growth, it was found necessary to clip off the apex of the bolls and split down the walls to expose the seeds. The removal of the seeds from the fresh bolls in either manner was a slow process. The seeds were dried in an electric oven at 96° C.

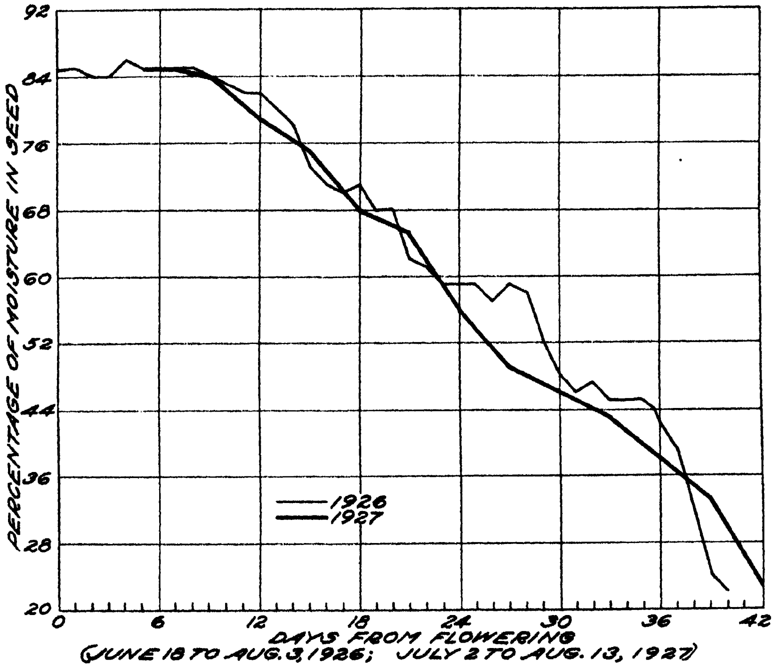

FIG. 5.—Percentage of moisture, based on wet weight of 100 seeds of Rio flax, at University Farm, St. Paul, Minn., determined daily in 1926 and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927

The percentage of oil was determined by the optical method as developed by Coleman and Fellows (*2*). This test is based on the difference in the refractive index of linseed oil and of a solvent known as halowax oil.

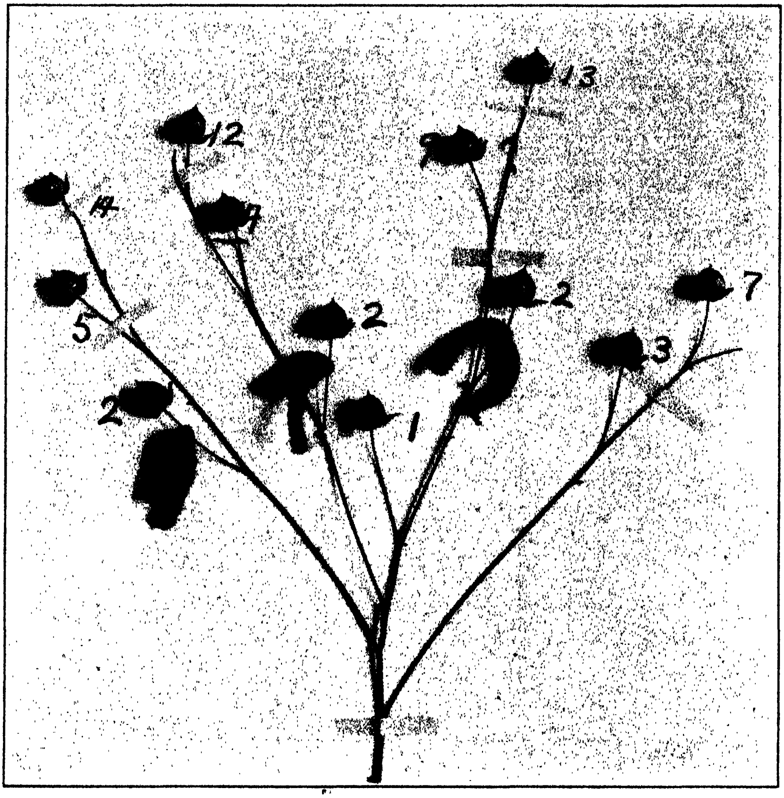

FIG. 6.—Panicle of Rio flax (C. I. 280) grown at University Farm, St. Paul, Minn., showing the dichotomous manner of branching and the actual date of blooming of all flowers (July 1-14). On July 2, 1927, more than 3,000 flowers were tagged to furnish seed of known age for the oil analyses

TABLE 4.—*Percentage of oil (on dry basis) of seeds of Rio flax at intervals after flowering to maturity at St. Paul, Minn., and at Mandan, N. Dak., in 1926 and 1927*

Time from flowering	St. Paul, 1926 [a]		St. Paul, 1927 [b]				Mandan, 1926 [c]		Mandan, 1927 [d]	
	Date of sampling	Oil	Early bloom		Late bloom		Date of sampling	Oil	Date of sampling	Oil
			Date of sampling	Oil	Date of sampling	Oil				
		P. ct.		*P. ct.*		*P. ct.*		*P. ct.*		*P. ct.*
5 days	------	------	July 7	0.83	------	------	------	------	July 19	1.40
7 days	------	------	July 9	1.10	------	------	------	------	July 21	2.39
8 days	July 1	1.84	------	------	------	------	------	------	------	------
9 days	July 2	2.38	July 11	2.50	------	------	July 26	17.42	July 23	5.41
12 days	July 6	8.15	July 14	8.73	July 24	5.40	July 29	27.74	July 25	13.92
15 days	July 9	26.00	July 17	19.42	July 27	19.82	Aug. 1	36.10	July 28	33.48
18 days	July 12	34.66	July 20	30.98	July 30	35.03	Aug. 4	38.40	July 30	37.61
21 days	July 10	35.56	July 23	39.00	Aug. 2	40.58	Aug. 7	40.72	Aug. 3	43.23
24 days	July 13	37.50	July 26	43.75	------	------	------	------	Aug. 5	44.60
27 days	July 16	40.25	July 29	43.97	------	------	Aug. 13	42.41	Aug. 8	44.57
30 days	July 19	40.75	Aug. 1	43.34	------	------	------	------	Aug. 11	43.93
33 days	July 27	40.72	Aug. 4	42.62	------	------	------	------	Aug. 14	43.01
36 days	July 30	40.56	Aug. 7	42.88	------	------	------	------	Aug. 17	42.48
39 days	Aug. 2	40.74	Aug. 10	42.92	------	------	------	------	------	------

[a] Flowers tagged on June 19, 22, and 24.
[b] Early bloom tagged July 2, late bloom tagged July 12.
[c] Flowers tagged July 17.
[d] Flowers tagged July 12, 13, and 14.

Two grams of each sample of seed were ground in a mortar with sand, the mortar being heated to about 70° C. to facilitate oil extraction. After the seed had been ground as fine as possible, 4 c. c. of halowax oil was added and the grinding continued for two or three minutes. The dissolved oil was then filtered through a paper filter, and the readings were made on the refractometer.

The oil content of the seeds on a dry basis is shown in Table 4 and graphically in Figure 7.

It will be seen by reference to Figure 7 that the percentage of oil in the growing flaxseed increased very rapidly for a period of about 15 to 18 days, that is, from about the seventh or ninth day after flowering to the twenty-first or twenty-seventh day. In this brief period the oil content increased from about 2 per cent to 40 per cent or more in the variety studied. After nearly the maximum percentage was reached, there was slight change up to full maturity. (Fig. 8.)

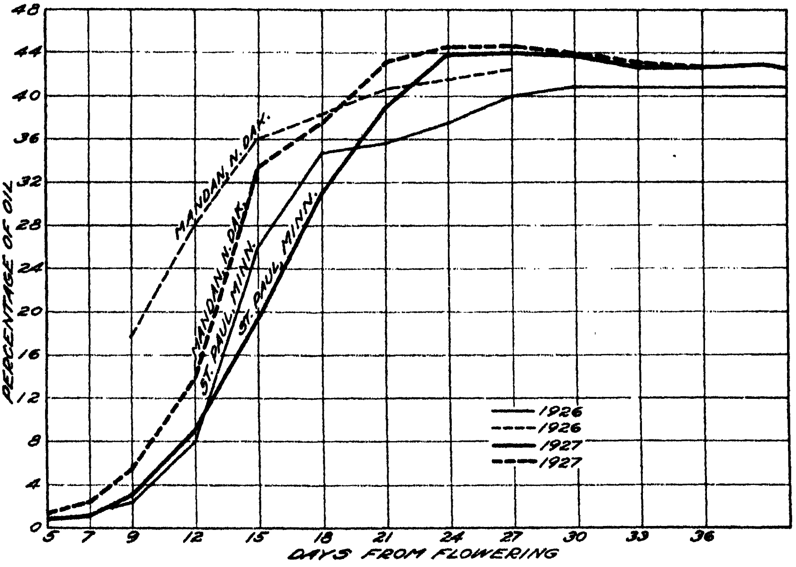

FIG. 7.—Percentage of oil (dry basis) in seeds of Rio flax (C. I. 280) at intervals from flowering to maturity at St. Paul, Minn., and at Mandan, N. Dak., in 1926 and 1927

There is an increase in the total oil content of the seeds, however, so long as there is an increase in dry weight. This is shown by reference to Figure 9, in which the total quantity of oil is depicted graphically as grams of oil in 1,000 seeds. The total quantity of oil is a product of the dry weight by the percentage of oil as determined at the indicated regular intervals after flowering. The graphs show that there is a rapid and very constant deposit of oil in the seeds from about the ninth day after flowering until the seeds are mature; that is, until the increase in dry weight is completed. This point was reached at about the thirty-sixth day at St. Paul, in both years, and at about the thirtieth day at Mandan, in 1927. The earlier development of the seeds at Mandan in 1926 is hereafter considered.

EFFECT OF DROUGHT AND TEMPERATURE ON SEED DEVELOPMENT

Reference to Figures 7 and 9 shows that the development of the seeds at Mandan was much more rapid in 1926 than in 1927. This was due to the extreme drought accompanied by high temperatures which prevailed at Mandan during the growing season of 1926. In both years the soil contained little moisture at seeding time. In 1926 the drought continued through the growing period, being especially severe during June and the first 18 days of July. In 1927 abundant rains occurred in May. Data on the rainfall at Mandan for each of the four months, April to July, in 1926 and 1927, and the 50-year average or normal for that locality are shown in Table 5.

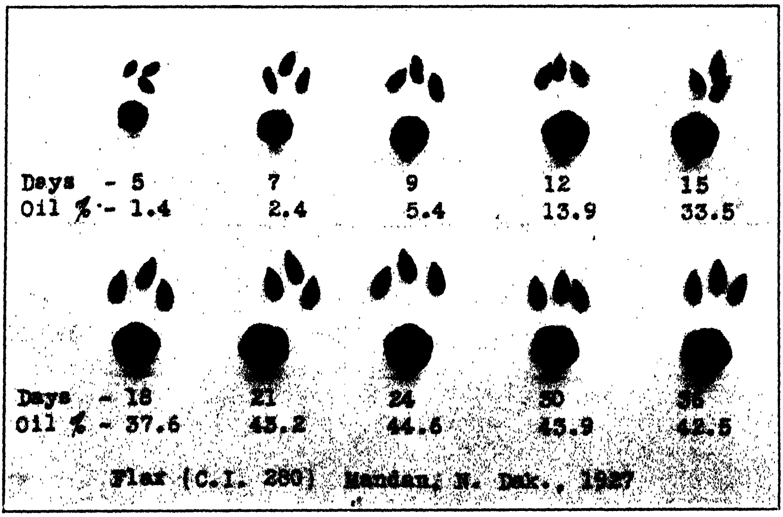

FIG. 8.—Illustration showing rapid development of bolls and seeds of Rio flax (C. I. 280) from about the seventh to the twenty-first days from flowering at Mandan, N. Dak., in 1927

TABLE 5.—*Precipitation at Mandan, N. Dak., for four months, April to July, in 1926 and 1927, compared with the 50-year average*

Month	Rainfall		
	1926	1927	Normal (50-year average)
	Inches	*Inches*	*Inches*
April	0.13	1.37	1.62
May	2.41	6.65	2.37
June	1.20	2.00	3.46
July	[a]2.19	2.37	2.29
Total	5.93	12.39	9.74

[a] The rainfall in July, 1926, nearly all occurred after July 18.

As a result of the drought in 1926, the plants were dwarfed, 10 to 14 inches high, without basal branches, and produced only three or four bolls per plant. Of more than 3,000 flowers tagged with yarn on the day of blooming less than 1,000 produced bolls. The others failed to develop, perhaps because of nonfertilization. In the bolls that formed there were few seeds. From 305 bolls 1,158 seeds were obtained, an average of 3.8 seeds per boll, as compared with 8.9 seeds per boll in the crop of 1927.

As is often the case, the drought of 1926 at Mandan was accompanied by periods of high temperatures. Figure 10 shows graphically the maximum and mean temperatures for the periods beginning 2 days before flowering and extending to 22 days after flowering in both 1926 and 1927. High temperatures prevailed before and after the date of flowering in 1926. The average daily maximum temperature for the 24-day period covered by the graph was 84.6° F. in 1926 and 79.4° in 1927, a difference of 5.2°. The average mean temperature for the period was 4° higher in 1926 than in 1927.

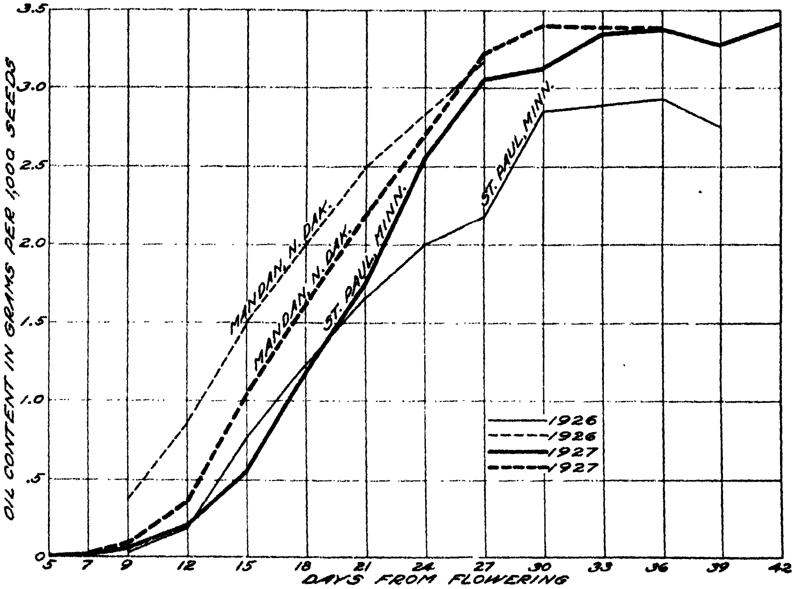

FIG. 9.—Total quantity of oil in 1,000 seeds of Rio flax at St. Paul, Minn., and at Mandan, N. Dak., at three-day intervals beginning on the ninth day in 1926, and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927

Drought and high temperatures, no doubt, caused the low fertility of the flax flowers. The high temperatures also probably account in part for the more rapid development of the seeds in 1926 than in 1927. Early maturity is the usual effect when drought occurs after the flowering period. Under conditions of drought the entire activity of the plant seems to function toward maturing the few bolls formed, resulting in the earlier deposit of oil as found in the 1926 crop at Mandan.

RATE OF SEED DEVELOPMENT FROM EARLY AND FROM LATE FLOWERS

In order to determine whether there was any material difference in the rate of development of seeds from the early flowers and from the late flowers on the same flax plants, flowers were tagged on July 2 (early flowers) and on July 12 (late flowers) at St. Paul in

1927. Bolls were collected at 3-day intervals during the period of most rapid oil formation as previously determined—namely, at 12, 15, 18, and 21 days after flowering—and the dry weight, the percentage of oil, and the total quantity of oil were determined. These data are given in Table 6.

Reference to Table 6 shows no consistent difference between the rate of development of the seeds from the early flowers and those from the late flowers as determined by the weight of 100 seeds. There were some variations in the percentage of oil in seeds of the same age and in the total quantity of oil, but these variations were not consistent and probably not significant. The entire growing season was favorable, however, and the plants were not checked in growth at any time, as is proved by the high yield of 26.3 bushels per acre obtained from the plots of this variety, so it is probable that no significant difference should be expected.

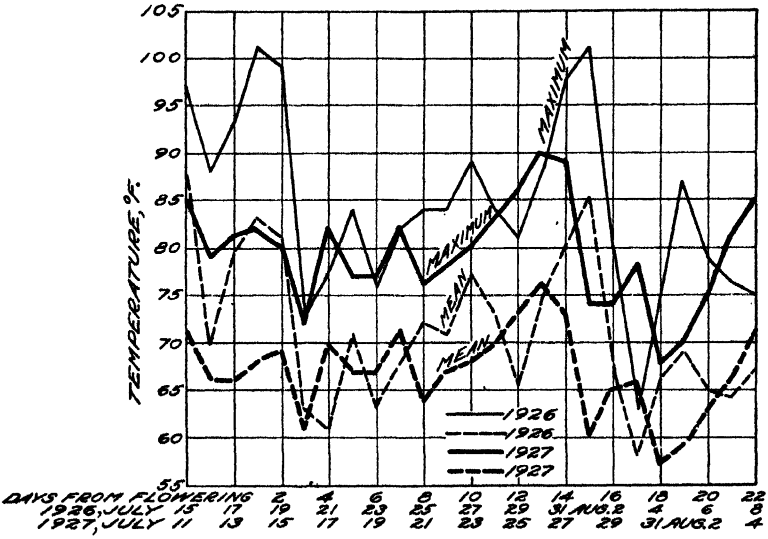

FIG. 10.—Maximum and mean daily temperature at Mandan, N. Dak., during periods of 24 days beginning 2 days before the flowering of flax and extending to 22 days after flowering, in 1926 and 1927

TABLE 6.—*Dry weight of 100 seeds, percentage of oil (dry basis), and total quantity of oil in 1,000 flax seeds obtained at St. Paul, Minn., from flowers tagged in the early-bloom period (July 2) and in the late-bloom period (July 12) on the same plants in 1927*

Time after flowering	Dry weight of 100 seeds		Percentage of oil in seeds		Total quantity of oil in 1,000 seeds	
	Early bloom	Late bloom	Early bloom	Late bloom	Early bloom	Late bloom
	Gram	*Gram*	*Per cent*	*Per cent*	*Grams*	*Grams*
12 days	0.2470	0.1977	8.73	5.40	0.216	0.106
15 days	.2890	.2900	19.42	19.82	.561	.575
18 days	.3832	.4026	30.98	35.03	1.187	1.410
21 days	.4456	.4550	[a] 39.00	40.58	1.738	1.846

[a] Interpolated from other determinations. (See fig. 7.)

It is possible, however, that a difference in the rate of oil deposition between the early-formed seeds and the late seeds would occur if there were a marked difference in temperature during the two periods of seed development. A period of higher temperatures for 10 to 20 days after the blossoming period would hasten the development of the late-formed bolls and seeds as compared with the early ones if the latter made their growth during a cool cloudy period. This fact was suggested by small differences observed in the rate of growth of bolls and seeds of the same age (time from flowering) but collected several days apart at St. Paul in 1926.

COMPARATIVE DEVELOPMENT OF SEEDS FROM ALL FLOWERS AND FROM EARLY FLOWERS

As mentioned under "Earlier investigations," the studies heretofore made on the oil content of immature flaxseed have been based on samples obtained by harvesting (pulling) and drying the plants and threshing out all the seeds for oil analysis. Such bulk samples are a mixture of seeds of various ages, depending upon the age of the individual bolls in days after flowering. A thousand seeds 5 days old have about the same mass (dry weight) as 100 seeds 24 days old. In order to compare the development of the bulk seeds with the seeds of known age in days, from the same or similar plants, typical plants were pulled at three-day intervals and dried in the laboratory. Later the yield of seed from 50 plants, the weight of 100 seeds representing a uniform sample, and the percentage of oil were determined. Data obtained at both St. Paul and Mandan in 1927 are presented in Table 7.

TABLE 7.—*Yield of seed per plant, weight of 100 seeds, and percentage of oil from flax plants harvested at three-day intervals from date of last blooming to maturity at St. Paul, Minn., and at Mandan, N. Dak., in 1927*

Place and date of harvest [a]	Stage of growth	Weight of— Seed per plant	Weight of— 100 seeds	Percentage of oil (dry basis)
St. Paul, Minn.:		*Grams*	*Gram*	
July 14	Date of last bloom; dry seeds very thin, green or colorless	0.06	0.142	23.54
July 17	Dry seeds still green or colorless, a few brown	.14	.227	29.68
July 20	Plants still green. Half of dry seeds brown, but very thin.	.19	.320	33.15
July 23	Plants green; over half of dry seeds brown, others green or colorless.	.20	.361	35.80
July 26	Plants green; a few ripe bolls; seeds brown, a few colorless.	.26	.547	39.61
July 29	Plants green; many ripe bolls; seeds brown, a very few thin.	.38	.589	40.52
August 1	Plants still green; most bolls ripe; seeds brown, some thin.	.38	.661	41.88
August 7	Plants partly brown, ripening; bolls ripe; seeds brown	.36	.740	40.09
August 10	Plants nearly ripe; seeds brown, plump	.45	.753	40.31
August 13	Plants fully ripe	.45	.772	40.03
Mandan, N. Dak:				
August 3	Date of last bloom; dry seeds very thin, green or colorless	.35	.291	33.85
August 6	Plants green; about half of seeds brown, others colorless	.53	.336	34.91
August 9	Plants green; nearly all seeds brown, but thin, a few colorless.	.53	.446	36.88
August 12	Plants green; most seeds brown, a few colorless	.80	.489	38.72
August 15	Plants ripening; many bolls brown; seeds all brown	.97	.640	41.30
August 18	Plants nearly ripe; most bolls ripe; seeds brown, a few thin and immature.	1.07	.660	41.47

[a] The first flowers appeared at St. Paul about June 28, first full bloom July 2, last bloom July 14; first flowers at Mandan about July 8, full bloom July 14, last bloom August 3.

The plants at St. Paul were grown in drilled plots (rows 7 inches apart) and were pulled from the second row from the border of the plot. The plants at Mandan were grown in cultivated rows 12 inches apart. This difference in culture accounts for the greater yield of seed per plant of the plants grown at Mandan.

At St. Paul the weight of 100 seeds increased from 0.142 gram on July 14, the date of last blooming, to 0.772 gram on August 13, when the plants were ripe. The proportion of oil increased from 23.54 per cent to 40.03 per cent during the same period. A similar development is shown in the samples from Mandan.

The seeds of uniform age, obtained by tagging flowers on the day of blooming, show a more rapid increase in oil content than the bulk seeds from all flowers. This is shown graphically in Figure 11.

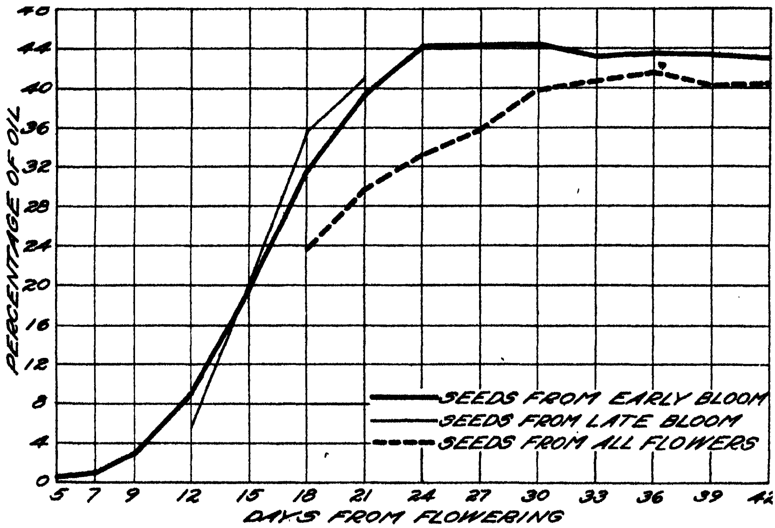

FIG. 11.—Percentage of oil in flaxseed from the early full bloom (flowers tagged July 2), from the late bloom (flowers tagged July 12), and from bulk seeds (all flowers). The first bulk sample was obtained at St. Paul, Minn., in 1927 by pulling the plants on July 14, about 18 days after the first flowers appeared

MICROCHEMICAL OBSERVATIONS OF PERIOD OF OIL FORMATION

Observations were made at St. Paul, by means of microchemical tests, of the changes that occur in the young seeds. The yellow stain, Sudan III, is specific for oil and was used in staining sections of seeds daily for several days after flowering.

In seeds three and four days after flowering, the tissues of the cotyledons contained many clear white grains or crystals, which stained black with iodine, indicating that they were starch grains. The integuments of the seed, the seed coat, also contained starch at this stage. No positive test for oil was obtained.

In seeds six days after flowering green chlorophyll was present in the cotyledons. The cotyledons stained distinctly with Sudan III, but stained only slightly with iodine. This would indicate that the starch which was present in seeds four days after flowering had been converted into oil. No tests for oil were obtained before chlorophyll appeared. A test for oil always was obtained where chlorophyll was present, the seeds staining distinctly with Sudan III.

These tests were merely preliminary, and it is planned to investigate further this phase of the subject.

The brown coloring matter of the seed coat, which appears in a single cell layer of the integuments, did not appear in the freshly gathered seeds until the seeds were nearly mature, that is, from the twenty-seventh or thirtieth day after flowering at St. Paul in 1926. The color apparently is present earlier and develops in seeds 12 to 18 days old as the wet seeds dry out, but it is not visible in the fresh seeds. The brown pigment is some form of tannin probably in combination with a resinous or fatty substance, presumably linseed oil, according to an unpublished report by Albert Mann, formerly of the Bureau of Plant Industry, United States Department of Agriculture.

GERMINATION OF IMMATURE FLAXSEED

The samples of seed collected at Mandan in 1927 were tested for germination. These seeds were obtained from bolls collected at three-day intervals after flowering and dried in a well-ventilated attic room. The germination test was made on moist blotters in a moist chamber kept at 20° C. at night and at 30° during the day.

At the end of 6 days no germination had occurred in seeds harvested at 9 or 12 days after flowering. There was 38 per cent germination in seeds harvested at 15 days, 80 per cent at 18 days, 90 per cent at 24 days, and an average of 95 per cent in four samples harvested at 27 to 36 days after flowering. A photograph of the germinated seeds is reproduced in Figure 12.

There was no germination of seeds harvested before 15 days after flowering. Seeds after 15 days' development, had attained about one-third of their mature dry weight and contained 33.48 per cent of oil. The fresh seeds, at 15 days, had reached their maximum growth in volume, the cotyledons were green with cholorophyll, and the embryo was formed completely.

DISCUSSION AND CONCLUSIONS

This paper presents data showing the daily growth of flaxseed of one variety, Rio (C. I. 280), from flowering to maturity at University Farm, St. Paul, Minn., in 1926, and of the time of oil formation in the developing seed as determined at St. Paul in 1926 and 1927 and at Mandan, N. Dak., during the same years. This is a subject of some interest as a study of plant metabolism and of practical importance in its bearing on the question of how early the flax crop can be harvested without loss in yield or quality of seed.

The data show that the growth of flaxseed, as determined by measurements of length, width, and thickness, is comparatively rapid. The seed increased proportionately in all three dimensions during the same period, reaching a maximum volume at 12 to 14 days after flowering. The volume remained more or less constant until the thirty-first day, after which a slight decrease occurred during the ripening stage. The growth of the flaxseed is different, therefore, from that of the barley kernel as determined by Harlan (*4*). In the barley kernel the growth in length is completed by the seventh day after flowering, the lateral diameter continues to increase to about the seventeenth day, and the dorsiventral diameter increases until the grain is nearly mature at 24 days after flowering.

The wet weight of 100 seeds, as would be expected, increased with the increase in volume and reached nearly its maximum at 13 days. (Fig. 4.) It decreased rapidly after the thirty-fifth day, during the ripening process.

The growth of the seed, as determined by the daily increase in dry weight of 100 seeds, continued uniformly for a period of 33 days. Thereafter the weight remained constant to the end of the ripening period at 39 or 40 days. This would indicate that there would not be any loss in total yield of seed if the crop were harvested somewhat "on the green side," that is, before the bolls and stems were fully brown.

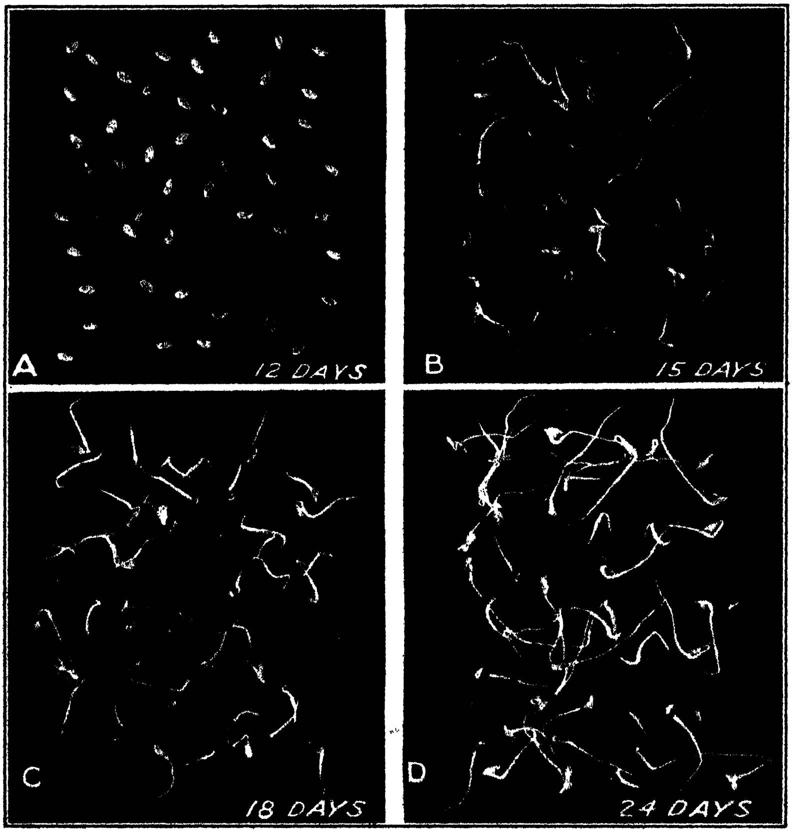

FIG. 12.—Germination of flaxseed harvested at Mandan, N. Dak., in 1927: A, 12; B, 15; C, 18; and D, 24 days after flowering

The most rapid formation of oil, based on the percentage of oil in the dry seeds, begins at about the seventh day after flowering and continues for a period of 15 to 18 days. After the maximum percentage is reached there is little or no significant change up to full maturity.

The total oil content of the seeds continues to increase with the increase in dry weight. The maximum oil content of the seeds is coincident, therefore, with the maximum dry weight. This point apparently is reached some six to nine days before the seeds are fully ripe and dry enough to harvest as usually practiced.

Where fiber flax is grown it is usually harvested somewhat green. Where flax is grown for seed, the straw often is used for the manufacture of coarse tow and sometimes as feed for livestock. For both of these purposes it is more valuable if harvested while the stems are still somewhat green. The data indicate that flax may be harvested before the plant is ripe and dry without sacrifice in weight of seed (that is, in yield per acre) or in percentage yield of oil.

Severe drought at Mandan in 1926 dwarfed the vegetative growth of the flax plants, caused fully two-thirds of the flowers to blight without setting bolls, and reduced the number of seeds per boll. The drought apparently hastened the time of oil deposition and of maturity as compared with the normal moisture conditions which prevailed in 1927. This may have been due partly, also, to the somewhat higher temperatures that prevailed at Mandan in 1926. The actual time when growth ceases must depend on the climatic conditions of temperature, humidity, and especially of soil moisture.

The brown color of the seed coat does not appear in the freshly gathered seed until the seed is nearly mature, that is, at approximately the time when the maximum dry weight is attained. This was at about the thirty-sixth day after flowering at St. Paul in 1926. The coloring substance, some form of tannin, evidently is present in the seed coat at an earlier stage, because it appears on drying the seeds collected at 12 to 18 days after flowering.

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PRELIMINARY NORMAL YIELD TABLES FOR SECOND-GROWTH WESTERN YELLOW PINE IN NORTHERN IDAHO AND ADJACENT AREAS[1]

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INTRODUCTION

In northern Idaho are large areas of forest land covered with dense, fairly uniform stands of second-growth western yellow pine (*Pinus ponderosa*), varying in age from a few years to 40 or 50 years. For the most part these young stands of yellow pine are found along the foothills of the great forests of the Bitterroot and Coeur d'Alene Mountains, fringing the borders of the fertile and thickly settled prairie lands to the west.

It is desirable to have reliable information upon the quantity of material which these stands will produce in a given period and to know the age at which owners should harvest the crop in order to realize maximum profit. Few stands elsewhere have developed under the conditions which have governed the growth of these young stands. Virgin western yellow pine is practically always many-aged; even-aged stands rarely develop except as a result of cutting. The oldest cuttings in this section are not over 50 years; and hence sources of information as to what these young stands will yield when they reach 70, 100, or 150 years of age are very meager.

The yield tables presented in this paper are compiled from the best material at present available. It is recognized that the results are not conclusive, because of the limited data upon which they are based; yet it is felt that they closely approximate conditions actually existing, and will serve a useful purpose until more comprehensive and reliable figures are available.

MATERIAL AND TECHNIC

Material for the study was obtained, during the author's connection with the University of Idaho, from 83 sample plots in pure even-aged western yellow pine, varying in size from 0.0625 to 0.75 acres. The smallest plots were confined to the younger age classes. The plots ranged from 30 to 163 years in age and represented some of the very poorest as well as some of the best timber-growing soils in the region. The plots were scattered from the vicinity of Moscow, Idaho, on the south, to near Newport, Wash., on the north, and from Fish Lake and a point about 9 miles west of Spokane, Wash., on the west, to Hayden Lake and Harvard, Idaho, on the east.

Because of the scarcity of extensive even-aged stands it was often necessary to take small plots in even-aged groups wherever they

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might be found. The boundaries of the plots were kept within the stand as far as possible, and whenever it was necessary to include the edge of a group in the plot a fair allowance was made for area actually occupied. Surveys were made with staff compass and tape, and, in order to eliminate to a large degree the errors of personal judgment in fixing arbitrary plot outlines, the plots were, with one or two exceptions, kept to a rectangular form.

On each plot a tally was made of all living trees down to 3 inches in diameter at breast height. Diameter and crown class were recorded for each tree. Heights covering the range of sizes present were measured on a sufficient number of trees (usually 8 to 15) to permit the drawing of a smooth curve of height on diameter for each plot or group of plots in the same stand. Form-point heights [2] were measured on 5 to 10 trees on each plot as the basis for assigning the plots to their proper form classes. Age was ascertained by increment borings or stump counts. These measurements were converted to total age by a special study of seedling development, which showed that on an average 4 years are required to reach a stump height of 1 foot and 10 years to reach 4.5 feet, the height at which increment borings were taken.

In the office the total cubic-foot volume of each plot was computed with the aid of form-class volume tables constructed by the author, and based in part upon sample trees cut from plots used in this study. These volume tables show entire wood content of the stem without bark, including tip but excluding a stump of 1 per cent of the total height and also butt swell above this point. Table 1 shows the volumes for form class 70,[3] which will be most generally used for stands less than 100 years of age. Board-foot volumes for each plot were calculated from Show's table for second-growth western yellow pine from the east side of the Sierras in California.[4] This table gives volumes according to the International log rule for ¼-inch saw kerf, with tops utilized to a diameter inside bark of 4 inches.

Other values computed for each plot include:

Average diameter at breast height.
Average diameter at breast height of dominant and codominant stand.
Average height.
Average height of dominant and codominant stand.
Total basal area.
Total number of trees.
Ratio of board-foot volume to cubic-foot volume.

Average heights corresponding to the average diameters for the stand in question were read from the curves of height on diameter. All the figures were reduced to a per acre basis. The technic of constructing the tables from the material was essentially the same as that developed by Bruce.[5]

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[2] The form point is defined as that point in the crown where the wind pressure may be considered as concentrated, which usually will correspond to the center of gravity of the area presented to the prevailing wind. The form-point height is expressed as a percentage of the total height of the tree and is measured very rapidly with the use of a Christen hypsometer divided into 10 equal divisions.

[3] In these tables the trees are classified not only by diameter and height but also by form quotient, which is defined as the ratio between the diameter at one-half the height above breast height and the diameter at breast height. The expression "form class 70" indicates that the form quotient is 0.70.

[4] MUNNS, E. N., and BROWN, R. M. VOLUME TABLES FOR THE IMPORTANT TIMBER TREES OF THE UNITED STATES. PART I. WESTERN SPECIES. p. 135, Table 101. Washington, [D. C.] 1925. (U. S. Dept, Agr., Forest Serv.).

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TABLE 1.—*Volumes*[a] *of western yellow pines of form class 70*[b]

Diameter breast high	Volume of tree whose height is—										
	20 feet	30 feet	40 feet	50 feet	60 feet	70 feet	80 feet	90 feet	100 feet	110 feet	120 feet
	Cu.ft.	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*	*Cu.ft.*
3 inches	0.4	0.6	0.8								
4 inches	.8	1.1	1.4								
5 inches	1.2	1.7	2.1	2.6	3.0						
6 inches	1.8	2.4	3.1	3.7	4.3						
7 inches	2.4	3.3	4.2	5.0	5.9						
8 inches		4.3	5.4	6.5	7.7	8.9					
9 inches		5.4	6.8	8.2	9.7	11.2					
10 inches			8.4	10.1	11.9	13.7	15.6				
11 inches				12.2	14.4	16.6	18.9				
12 inches				14.4	17.0	19.7	22.4	25.2			
13 inches				16.8	19.9	23.0	26.2	29.3			
14 inches				19.5	23.0	26.6	30.3	33.8			
15 inches				22.3	26.4	30.5	34.7	38.7	42.9	47.0	
16 inches				25.3	30.0	34.6	39.4	44.0	48.6	53.5	
17 inches				28.5	33.7	39.0	44.4	49.5	54.8	60.2	
18 inches				32.0	37.7	43.6	49.7	55.3	61.4	67.4	
19 inches				35.5	42.0	48.5	55.3	61.5	68.2	75.0	
20 inches				39.2	46.3	53.6	61.1	68.0	75.4	83.0	90.2
21 inches						59.0	67.4	74.9	83.0	91.5	99.5
22 inches						64.7	74.0	82.2	91.0	100.5	109.0
23 inches						70.7	80.8	89.8	99.5	110.0	119.0
24 inches						76.9	88.0	97.6	108.0	119.0	129.0
25 inches						83.2	95.2	106.0	117.0	129.0	140.0
26 inches								114.0	126.0	139.0	151.0
27 inches								123.0	136.0	150.0	163.0
28 inches								132.0	146.0	161.0	175.0
29 inches								141.0	156.0	172.0	187.0
30 inches								151.0	167.0	184.0	200.0

[a] Based on taper curves by formula $y=\frac{x}{a+bx}$, in which x = distance from tip expressed as percentage of total height above breast height, and y = diameter at distance x from tip expressed as percentage of normal diameter at breast height. Volumes include full stem inside bark from tip to stump of 1 per cent of total height, excluding butt swell.

[b] Form class 70 is generally applicable in second-growth timber less than 100 years old of average density. For open-grown timber reduce values 8 per cent, and for very dense stands or timber over 100 years old increase values 8 per cent.

SITE CLASSIFICATION

Average height of dominant and codominant trees at 100 years of age was used as the basis for site classification. The site index for each plot was determined by reference to a series of anamorphic curves of average height of dominant and codominant trees on total age. In constructing these curves the graduating curve was obtained from plots which, from a preliminary trial, were found to fall approximately between site indices 70 and 100, constituting the most densely populated middle portion of the band of points. The plots representing the extremes in either direction were not included, because it seemed that their distribution was not at all regular over the entire range of ages, optimum sites being found only in younger age classes and poorest sites only in older ages. The site classification curves, showing the distribution of the material by ages and sites and illustrating the technic of anamorphosis used, are given in Figure 1.

The graduating curve shown by the heavier broken line was first drawn to balance the average points of the plots which would fall approximately between sites 70 and 100. The average points and their weights are indicated by triangles. A straight line (*a*) was drawn from the origin to intersect the graduating curve at any convenient point near its outer extremity. A horizontal line (*b*) was drawn from the intersection of the graduating curve and the 100-

year abscissa, taken as the basis for the site classification, to the radial line (*a*), which was also intersected at this point by a vertical line (*c*), representing the anamorphic position of the 100-year abscissa. A series of straight lines radiating from the origin were then drawn to intersect the anamorphic 100-year abscissa (*c*) at even 10-foot intervals. Only the radiant for the 40-foot site index is shown in the figure. To locate the various site-index curves, vertical lines are drawn for a number of ages, similar to *c*. The intersections of these new age verticals with the site-index radiants are carried over horizontally to the original age verticals, thus defining the points for the final curves. The construction lines, *b*, *c*, *d*, and *e*, *f*, *g*, illustrate

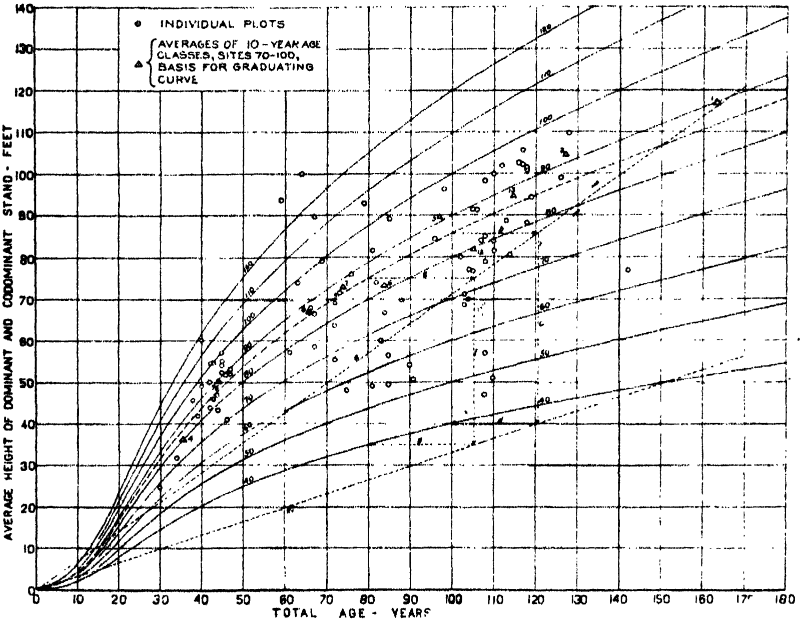

FIG. 1.—Site-classification curves of second-growth western yellow pine. Average height of dominant stand at various ages

the procedure for the 100 and 80 foot points and the 40-foot site-index curve.

BASAL-AREA CURVES AND REJECTION OF ABNORMAL PLOTS

Since total basal area per acre is considered the best basis for testing density of stand, a preliminary series of curves showing basal area per acre for each age and site class was required. Anamorphosis was again used, taking as the basis for the graduating curve only those plots falling between sites 70 and 100. The total basal area of each plot was then compared to the total basal area indicated by these preliminary curves for the given age and site in order to decide whether any of the plots were so abnormal as to justify rejection. Twice the standard error was considered the limit of variability, and this gave a range of about 48 per cent in either direction. Although there were several plots deviating approximately this much from the preliminary curves, none exceeded it by a significant amount, and it was decided to retain all the plots in the subsequent calculations.

TABLES FOR ENTIRE STAND

Anamorphic curves were drawn showing total basal area per acre, total number of trees per acre, basal area of average tree, total cubic foot volume per acre, and average height of entire stand. For each

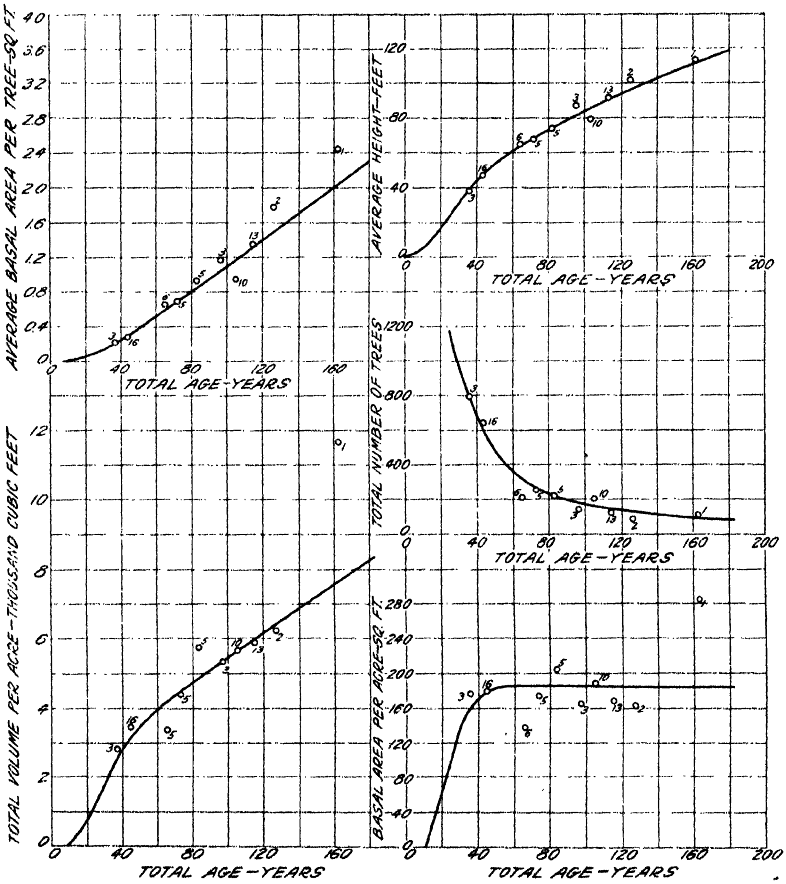

FIG. 2.—Graduating curves for yield tables of second-growth western yellow pine

of these factors the graduating curve was based on the 64 plots between sites 70 and 100. The graduating curves for the various factors were made to check with each other by making the curve for total basal area equal to the product of the curves for basal area of average tree and total number of trees at all ages. The curve for total cubic-foot volume was then made to check with values for total number of trees per acre multiplied by volumes corresponding to trees of average basal area and height from the curves. The graduating curves are shown in Figure 2.

The spacing of the curves for the various site classes about the graduating curves was accomplished by means of "intercept curves." As a basis for these the entire body of data was grouped into 10-foot site classes. Then for each plot a value for each factor was read from the graduating curves corresponding to the age of the plot. For each site group the actual values of each factor were then totaled and divided by the sums of the values read from the graduating curve. These percentage ratios were then plotted over site and smoothed intercept curves were drawn, in each case passing through 100 per cent at site 86.8, which represents the average site index of the plots upon which the graduating curves were based. The intercept curves were made to check among themselves in the same manner as the graduating curves. The check on the volume curve was made by using values for the 100-year age. A check of any single-age class establishes a check for the entire range, because the graduating curves, based on age, are already balanced throughout their length. The intercept curves are shown in Figure 3.

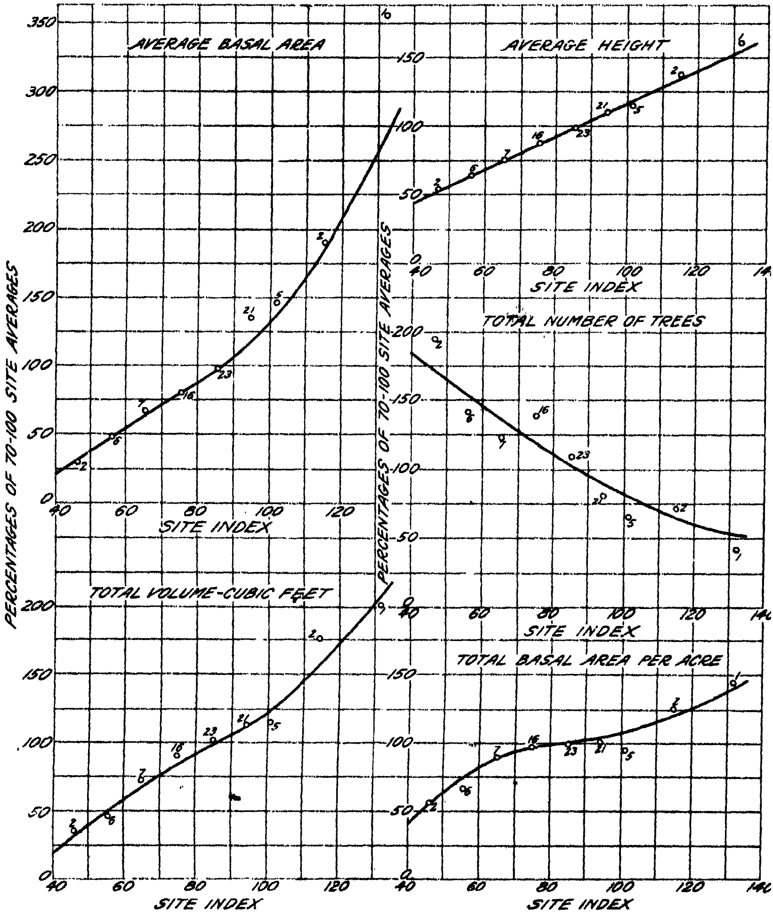

FIG. 3.—Intercept curves for yield tables of second-growth western yellow pine

By applying the percentages from the intercept curves to the graduating curves, the final series of curves showing values for the various factors on age, by site classes in the conventional form, were obtained. These are shown in Figures 4 to 7 and Tables 2 to 6.

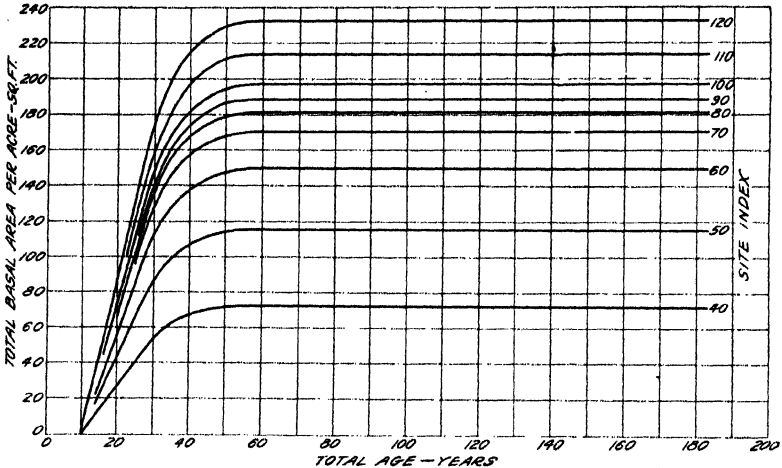

FIG. 4.—Total basal area per acre of second-growth western yellow pine at various ages

TABLE 2.—*Total basal area per acre of western yellow pine*

Age	Basal area of trees at site index indicated								
	40	50	60	70	80	90	100	110	120
	Sq. ft.	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*	*Sq. ft.*
30 years	55	88	114	130	138	143	150	163	178
40 years	67	107	138	158	169	175	183	198	216
50 years	71	114	147	168	179	186	195	211	230
60–180 years	72	115	149	170	181	188	197	213	232

TABLE 3.—*Total number of trees per acre of western yellow pine*

Age	Number of trees at site index indicated								
	40	50	60	70	80	90	100	110	120
30 years	1,800	1,614	1,438	1,252	1,080	929	792	675	582
40 years	1,246	1,117	995	867	751	643	548	467	403
50 years	892	800	713	621	538	461	393	335	289
60 years	660	592	528	460	398	341	291	248	214
70 years	521	467	416	362	314	269	229	195	168
80 years	427	383	341	297	258	220	188	160	138
90 years	361	323	288	251	218	186	159	135	89
100 years	311	279	248	216	188	160	137	117	98
110 years	274	244	217	189	162	140	118	100	85
120 years	243	218	194	169	146	125	107	91	78
130 years	221	198	176	154	133	114	97	83	71
140 years	202	182	162	141	122	105	89	76	65
150 years	188	168	150	130	113	97	83	70	61
160 years	177	158	141	123	107	91	78	66	57
170 years	166	148	132	115	100	86	73	62	53
180 years	156	140	125	109	94	81	69	59	50

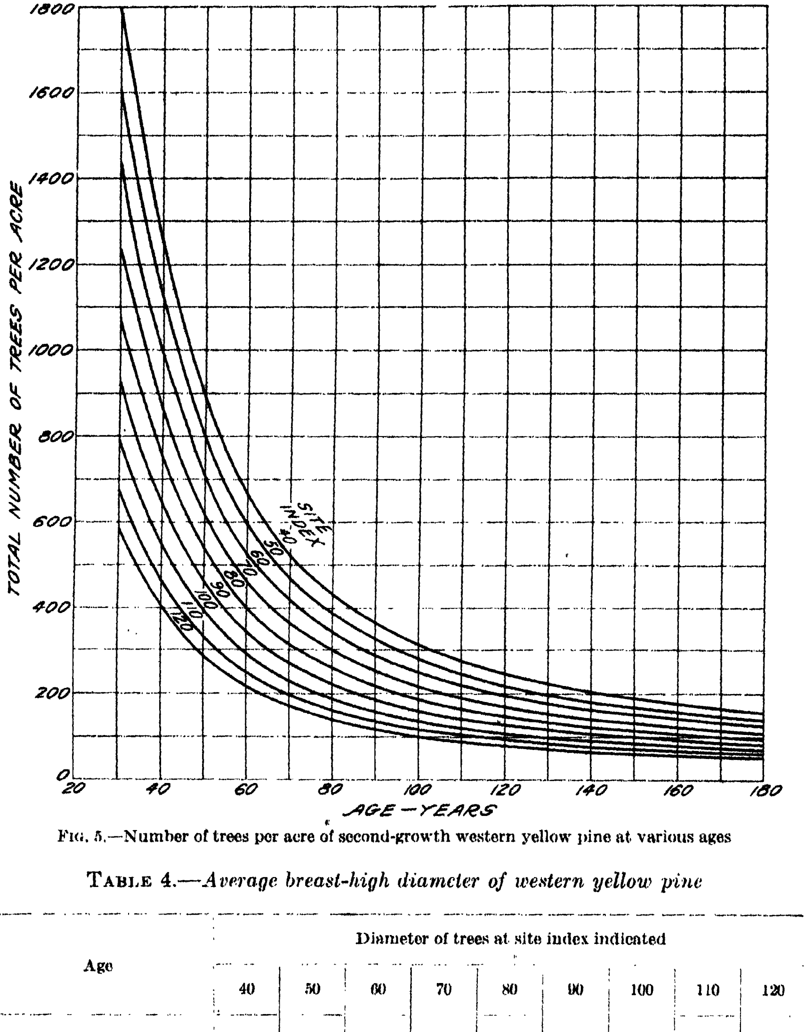

FIG. 5.—Number of trees per acre of second-growth western yellow pine at various ages

TABLE 4.—*Average breast-high diameter of western yellow pine*

Age	Diameter of trees at site index indicated								
	40	50	60	70	80	90	100	110	120
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
30 years	2.4	3.2	3.8	4.4	4.9	5.3	5.9	6.7	7.5
40 years	3.2	4.2	5.0	5.8	6.4	7.1	7.8	8.8	9.8
50 years	3.8	5.1	6.2	7.1	7.8	8.6	9.6	10.8	12.0
60 years	4.4	5.9	7.1	8.2	9.1	9.9	11.1	12.5	14.0
70 years	5.0	6.7	8.1	9.3	10.3	11.3	12.6	14.2	15.9
80 years	5.6	7.4	8.9	10.2	11.4	12.5	13.9	15.6	17.6
90 years	6.1	8.1	9.8	11.2	12.4	13.6	15.1	17.0	19.1
100 years	6.5	8.7	10.5	12.0	13.3	14.6	16.2	18.3	20.5
110 years	6.9	9.2	11.1	12.8	14.1	15.6	17.3	19.5	21.8
120 years	7.3	9.8	11.8	13.5	15.0	16.5	18.3	20.6	23.1
130 years	7.7	10.3	12.4	14.2	15.7	17.3	19.2	21.7	24.3
140 years	8.0	10.8	13.0	14.8	16.5	18.1	20.1	22.7	25.4
150 years	8.4	11.2	13.5	15.5	17.2	18.9	21.0	23.6	26.5
160 years	8.7	11.6	14.0	16.1	17.8	19.6	21.8	24.6	27.6
170 years	9.0	12.1	14.6	16.7	18.5	20.4	22.6	25.5	28.5
180 years	9.3	12.5	15.0	17.2	19.1	21.0	23.3	26.3	29.5

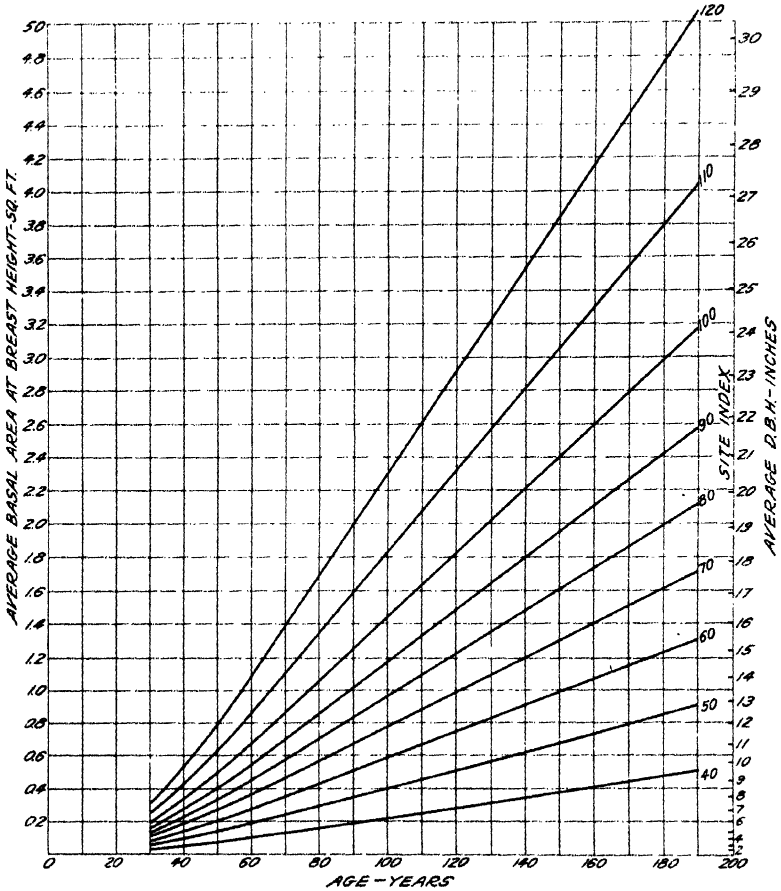

FIG. 6.—Average size of second-growth western yellow pine at various ages

TABLE 5.—*Average height of stand of western yellow pine*

Age	Height of trees at site index indicated								
	40	50	60	70	80	90	100	110	120
	Feet	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*
30 years	13	17	20	24	28	31	35	38	42
40 years	18	24	29	34	39	44	49	54	59
50 years	23	29	35	41	47	54	60	66	72
60 years	26	33	40	47	54	61	68	76	83
70 years	29	37	45	53	61	69	77	84	92
80 years	32	40	49	58	66	75	84	92	101
90 years	34	43	53	62	71	81	90	99	108
100 years	36	46	56	66	76	86	96	106	116
110 years	38	49	59	70	80	90	101	111	122
120 years	40	52	63	74	85	96	107	118	129
130 years	42	54	65	77	88	100	111	123	134
140 years	44	56	68	80	92	104	117	129	141
150 years	46	59	71	84	97	109	122	134	147
160 years	48	61	74	87	100	113	126	140	153
170 years	50	63	77	90	104	118	131	145	158
180 years	51	66	80	94	108	122	136	150	164

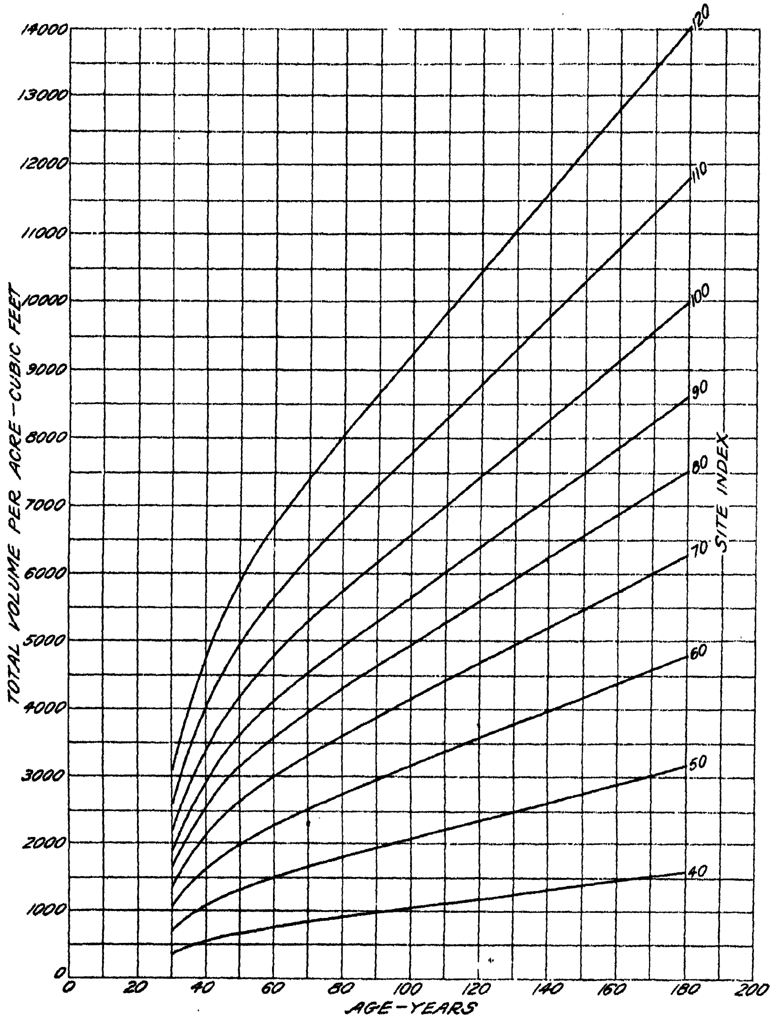

FIG. 7.—Yield per acre in cubic feet of second-growth western yellow pine at various ages

TABLES FOR TOTAL VOLUME IN BOARD FEET

The table showing volume in board feet was derived from the table for total cubic-foot contents by means of the ratios between board-foot and cubic-foot contents. The board foot-cubic foot ratios for all the plots were plotted over average d. b. h.[6] of the stand. This gave a narrow, well-defined band of points through which a smooth curve was drawn. (Fig. 8.)

The ratio for each age and site class was then read from this curve corresponding to the average diameters previously determined. These ratios were applied to the table of total cubic-foot volumes to get the final table of board-foot volumes. The results are shown in Figure 9 and Table 7.

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[6] Diameter at breast height (4½ feet).

TABLE 6.—*Total volume per acre in cubic feet of western yellow pine*

Age	Volume per acre of trees at site index indicated								
	40	50	60	70	80	90	100	110	120
	Cu. ft.	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*
30 years	350	700	1,050	1,380	1,650	1,880	2,190	2,590	3,080
40 years	550	1,080	1,630	2,140	2,560	2,920	3,400	4,020	4,780
50 years	670	1,330	2,010	2,630	3,150	3,600	4,190	4,950	5,880
60 years	770	1,520	2,290	3,000	3,590	4,110	4,780	5,650	6,720
70 years	850	1,680	2,530	3,310	3,970	4,540	5,280	6,230	7,410
80 years	920	1,820	2,750	3,600	4,310	4,930	5,740	6,780	8,060
90 years	990	1,960	2,960	3,880	4,640	5,300	6,170	7,290	8,670
100 years	1,060	2,100	3,160	4,160	4,960	5,670	6,580	7,790	9,270
110 years	1,130	2,240	3,370	4,420	5,290	6,040	7,010	8,300	9,860
120 years	1,200	2,370	3,570	4,680	5,610	6,410	7,430	8,810	10,450
130 years	1,270	2,510	3,780	4,950	5,920	6,770	7,860	9,310	11,050
140 years	1,340	2,640	3,980	5,210	6,240	7,130	8,280	9,810	11,640
150 years	1,410	2,780	4,180	5,480	6,560	7,500	8,720	10,310	12,240
160 years	1,470	2,910	4,380	5,750	6,880	7,860	9,140	10,810	12,830
170 years	1,540	3,040	4,580	6,010	7,190	8,220	9,560	11,300	13,430
180 years	1,610	3,170	4,780	6,270	7,510	8,580	9,980	11,800	14,020

TABLE 7.—*Total volume per acre in board feet of western yellow pine*

Age	Volume per acre in board feet of trees at site index indicated [a]								
	40	50	60	70	80	90	100	110	120
30 years			140	940	1,850	2,950	4,710	7,200	10,700
40 years		560	2,120	4,260	6,580	9,200	12,900	18,400	25,600
50 years	100	1,810	4,660	8,310	11,910	15,910	21,300	28,600	37,300
60 years	560	3,270	7,330	12,200	17,100	21,800	28,400	36,600	46,300
70 years	1,100	4,750	10,100	16,100	21,900	27,300	34,300	43,000	53,700
80 years	1,610	6,280	12,700	19,800	26,100	31,900	39,300	48,800	60,500
90 years	2,280	7,800	15,300	23,100	29,900	36,000	43,800	54,200	67,000
100 years	2,810	9,340	17,700	26,100	33,200	39,700	48,100	59,400	73,400
110 years	3,400	10,900	20,000	29,000	36,500	43,400	52,500	64,600	79,900
120 years	4,000	12,300	22,100	31,600	39,700	47,000	56,800	70,000	86,500
130 years	4,670	13,800	24,300	34,200	42,700	50,600	61,100	75,200	93,000
140 years	5,320	15,200	26,100	36,700	45,700	54,200	65,300	80,600	99,300
150 years	5,920	16,500	28,000	39,300	48,900	57,800	69,600	85,800	105,900
160 years	6,590	18,000	30,000	41,800	51,900	61,400	74,000	91,100	112,300
170 years	7,280	19,200	31,900	44,200	55,000	64,900	78,300	96,500	118,900
180 years	7,950	20,600	33,800	46,800	58,100	68,600	82,600	101,700	125,500

[a] International rule, ¼-inch saw kerf. Tops utilized to diameter of 4 inches inside bark.

MEAN ANNUAL GROWTH

The mean annual growth in cubic feet and board feet per acre was computed by dividing the figures for total volume by their respective ages. The results are given in Figure 10 and Tables 8 and 9. Mean annual growth affords the most satisfactory index of maturity of the timber by indicating the age at which the average annual growth per acre reaches a maximum. When total volume in cubic feet, regardless of size or quality, is considered, the maximum production is reached between the ages of 43 and 45 years on all sites, with the decline quite slow for the next 20 years. The poorest sites at 40 years of age average only 14 cubic feet per acre per year, while the best sites yield 120. Since the board-foot measure depends upon size as well as total wood volume per acre, and since commercial value is also dependent in large measure on size, the figures for mean annual growth in board feet are of more significance as a guide to management.

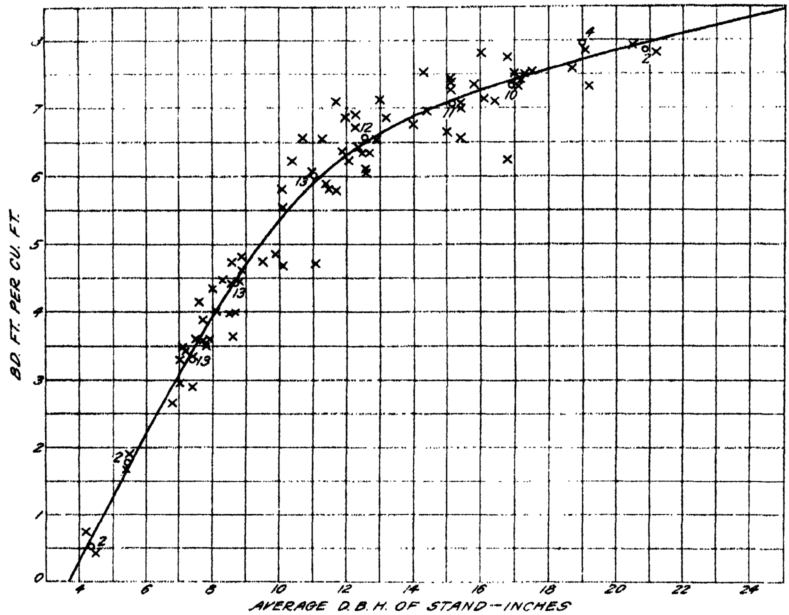

FIG. 8.—Number of board feet per cubic foot of western yellow pine in stands of various average diameters

Maximum mean annual growth is attained on site 120 at 60 years of age, and the period of culmination lengthens as the sites grow poorer, being 150 years for the 60-foot site class and failing to culminate within the limits of the data for the two poorest site classes. On the average site (between 80 and 90) the age of culmination is between 90 and 100 years and this is the period indicated as the most desirable age at which to cut second-growth western yellow pine.

TABLE 8.—*Mean annual growth in cubic feet of western yellow pine*

Age	Mean annual growth in cubic feet per acre at site index indicated								
	40	50	60	70	80	90	100	110	120
30 years	12	23	35	46	55	63	73	86	103
40 years	14	27	41	54	64	73	85	100	120
50 years	13	27	40	53	63	72	84	99	118
60 years	13	25	38	50	60	68	80	94	112
70 years	12	24	36	47	57	65	75	89	106
80 years	12	23	34	45	54	62	72	85	101
90 years	11	22	33	43	52	59	69	81	96
100 years	11	21	32	41	50	57	66	78	93
110 years	10	20	31	40	48	55	64	76	90
120 years	10	20	30	39	47	53	62	74	87
130 years	10	19	29	38	46	52	61	72	85
140 years	10	19	28	37	45	51	59	70	83
150 years	9	19	28	37	44	50	58	69	82
160 years	9	18	27	36	43	49	57	68	80
170 years	9	18	27	35	42	48	56	67	79
180 years	9	18	27	35	42	48	55	66	78

TABLE 9.—*Mean annual growth in board feet of western yellow pine*

Age	Mean annual growth in board feet per acre at site index indicated [a]								
	40	50	60	70	80	90	100	110	120
30 years			4.5	31.2	61.6	98.4	157	240	358
40 years		14	53	106	164	230	322	461	639
50 years	2	36	93	166	238	318	426	572	748
60 years	9	54	122	203	284	363	473	610	*772*
70 years	16	68	144	230	312	389	490	*615*	768
80 years	20	78	157	248	326	399	*491*	610	756
90 years	25	87	168	257	332	*400*	487	602	745
100 years	28	93	177	261	*332*	397	481	593	734
110 years	31	99	182	−264	332	394	477	587	726
120 years	34	103	184	*264*	330	391	473	583	720
130 years	36	106	187	+262	328	389	470	578	715
140 years	38	108	−188	262	326	387	466	574	710
150 years	40	111	+*188*	262	325	385	464	+571	706
160 years	41	112	188	262	324	384	+462	570	702
170 years	43	113	188	262	−324	382	460	568	700
180 years	44	114	188	262	323	381	459	565	697

[a] International rule, ¼-inch saw kerf. Tops utilized to diameter of 4 inches inside bark.

The maximum mean annual growth increases from 188 board feet per acre per year on the 60-foot site class to 772 board feet per acre per year for site index 120.

ALIGNMENT-CHART YIELD TABLE

The data in all the tables described above may be presented in compact graphical form for practical use by means of a single system of alinement charts devised by Reineke.[7] Such a chart is shown in Figure 11. The possibility of using this system depends on the fact that values for the various sites represent constant percentages of the average graduating curves for all ages.

On the left of the chart is a series of six scales for age and on the right a corresponding series for site index. Values are read from the central axis for any of the factors by joining the proper age point on a given scale on the left with the proper site-index point on the corresponding scale on the right. Readings on the central scale must be multiplied by 10 for cubic-foot volumes. Board-foot values are not shown directly, but the board foot-cubic foot ratios are obtained by drawing a straight line from the fixed point "P" near the right margin through the average d. b. h. on the central axis, to the scale of board feet per cubic foot. This ratio must then be applied to the figure for total cubic-foot volume.

STAND-TABLE GRAPH

In addition to figures showing total yield and average size, it is of value to be able to estimate the proportion of the total number of trees which will be above any given size, so that value of the yield in terms of special products, such as poles and posts or of timber of specific sizes, can be calculated.

Studies by Baker[8] and Bruce[9] indicate that in even-aged stands the trees are distributed among the different diameter classes in a consistent and definite manner for each species. For some species the distribution follows the normal frequency distribution, which is susceptible to definite mathematical treatment. Tests made with the data from the sample plots used in this study indicate that this is true in general for western yellow pine. Bruce has found that the distribution of the various diameter classes about the average is primarily dependent upon the average diameter of the stand, so that this is made the basis for the graphs rather than age and site. Special

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[7] REINEKE, L. H. A MODIFICATION OF BRUCE'S METHOD OF PREPARING TIMBER-YIELD TABLES. Jour. Agr. Research 35: 843–856, illus. 1927.

[8] BAKER, F. S. NOTES ON THE COMPOSITION OF EVEN-AGED STANDS. Jour. Forestry 21: 712–717, illus. 1923.

[9] BRUCE, D. Op. cit.

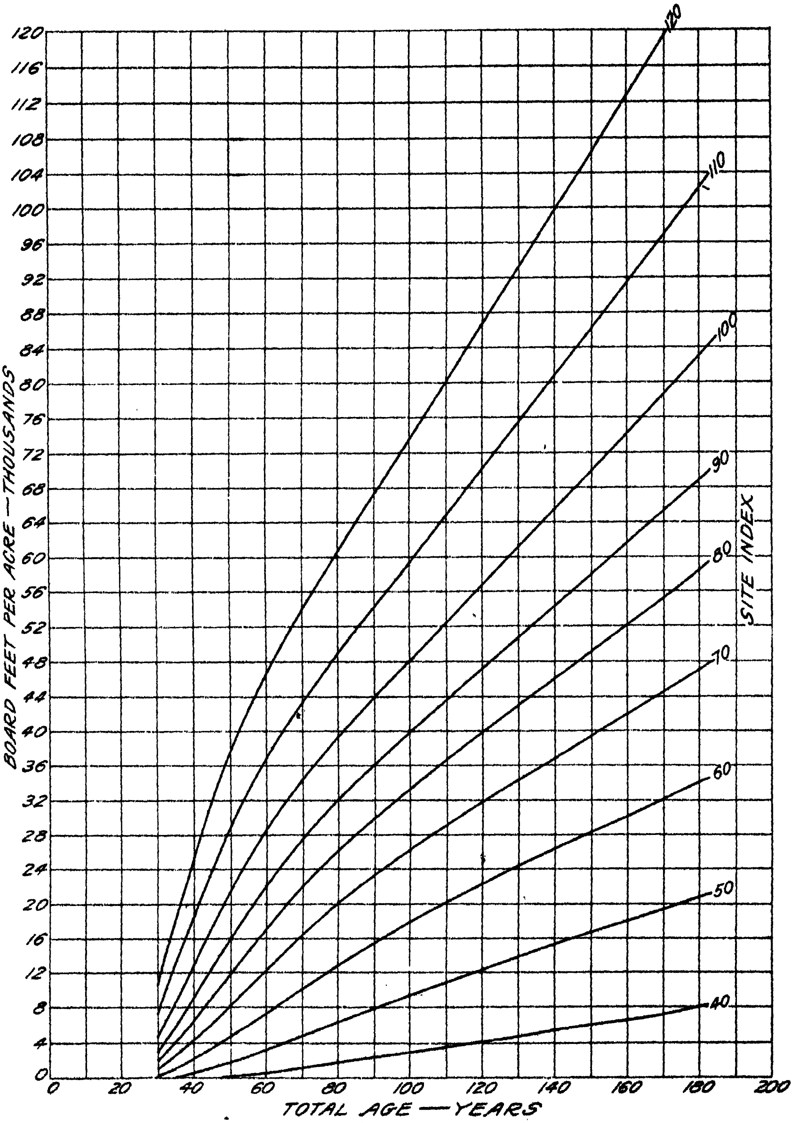

FIG. 9.—Yield per acre at various ages of second-growth western yellow pine in board feet. International rule, ¼-inch saw kerf. Trees 8 inches d. b. h. and up

coordinate paper is used on which any distribution conforming to the normal frequency will plot as a straight line, the slope of which indicates whether the scatter is great or small.

The plots were first grouped according to their average diameters into 2-inch classes, and for each group a composite stand table was made up. The composite stand tables were then expressed on a cumulative percentage basis, i. e., per cent of total number of trees up to 4 inches, 6 inches, 8 inches, etc. These percentages were

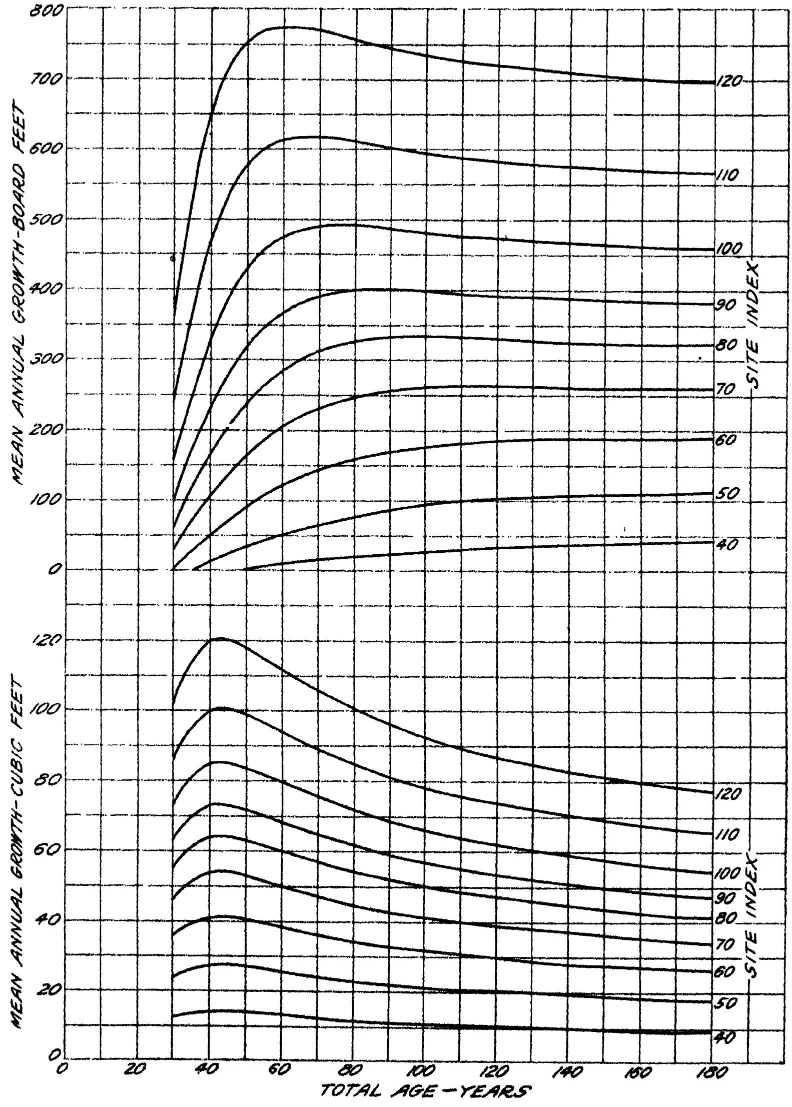

FIG. 10.—Mean annual growth of second-growth western yellow pine in cubic feet and board feet, International rule, ¼-inch kerf

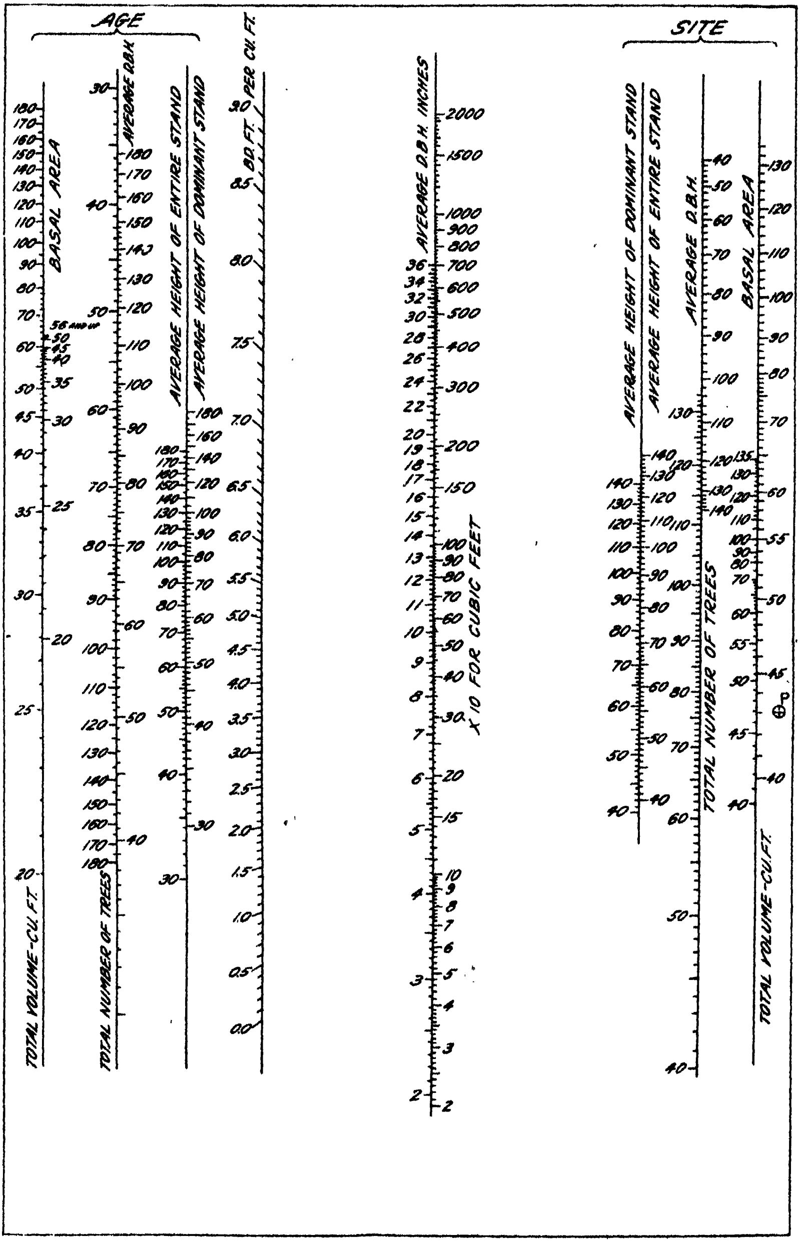

FIG. 11.—Alignment-chart yield table for second-growth western yellow pine. Values for total volume in cubic feet, total basal area per acre, total number of trees per acre, average d. b. h., average height of entire stand, or average height of dominant stand, can be read on central axis by a straight line joining the point for given age on any scale at the left with point for given site index on corresponding scale at right. Board foot-cubic foot ratios are read on board foot per cubic foot axis by a line from fixed point "P" through average d. b. h. for stand in question on central axis

plotted on frequency paper and straight lines fitted to them. The resulting series of lines was harmonized by plotting the intercepts on the 2 per cent and 98 per cent ordinates over actual average diameter of the stand groups and curving the results. From these curves intercepts on the 2 per cent and 98 per cent ordinates can be read off for the even-inch classes, and these values are entered on the final chart. (Fig. 12.) Straight lines joining the intercepts for any diameter class on these two scales represent the frequency distribution of the stands in question.

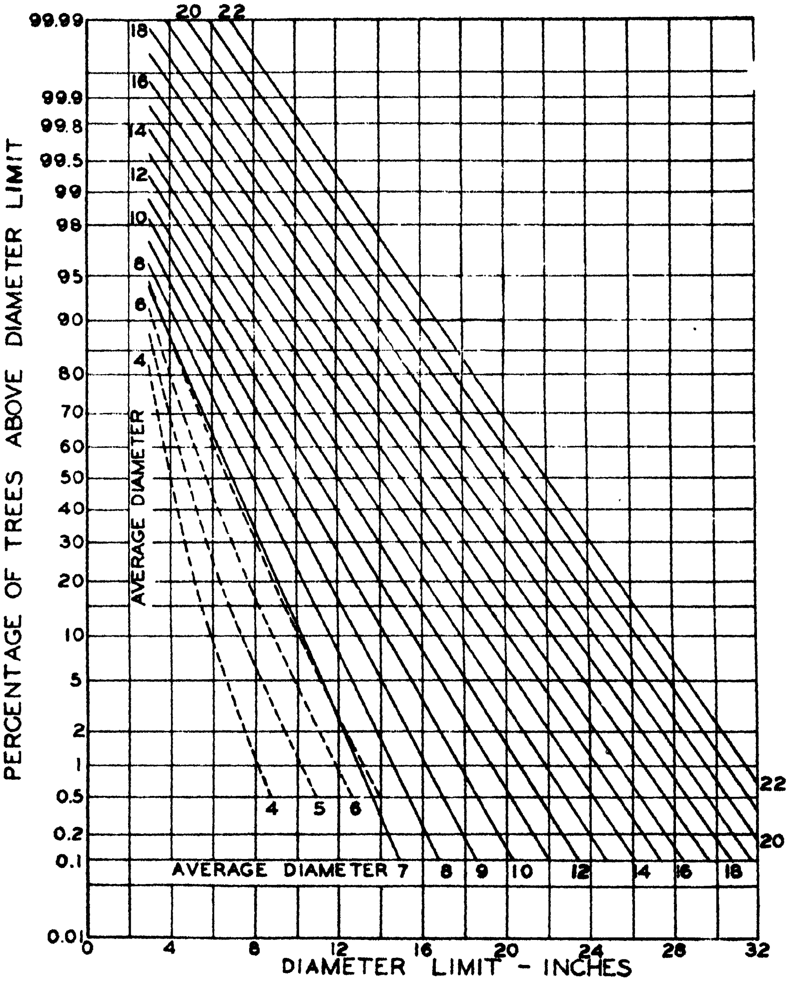

FIG. 12.—Stand-table graph for second-growth western yellow pine, showing proportion of total number of trees above given diameter limit according to the average diameter of the stand

The straight-line form failed to fit the data in stands below 7 inches in average diameter. In the stands of smaller size there is apparently a higher proportion of the trees below the average than in a normal frequency distribution. This is doubtless due, in part at least, to the fact that in the field no trees below the 3-inch d. b. h. class were included in the tally. In order to make the chart complete, the distributions for stands averaging less than 8 inches in diameter have been shown as curves, which may be applied with safety to stand tallies in which count is made only of trees 3 inches and over in diameter. The graphical method of presenting stand tables used here was devised by Reineke.[10]

DISCUSSION

It must be borne in mind that the yields shown in these tables represent what may be obtained for areas fully stocked with trees, and that in practice such fully stocked stands are never found over large areas. Yields of large areas are obtained by reducing the tabulated yields in proportion to the degree of stocking of the area in question. At the same time, the tables give conservative figures for fully stocked stands and by no means represent the maximum production which may be obtained under complete protection and careful management. Hardly any of the older plots on which this study is based have been free from fire throughout their lives. Many of them show evidence of having been burned over several times. The effect of fires is to thin out the numbers and at the same time reduce the rate of growth for many years of trees left alive. Thus, could the loss from frequent burning be eliminated, higher yields might be expected at the older ages. Insects and disease, which might be kept at a minimum in a managed forest, have also worked uncontrolled throughout the lives of the stands measured, and evidence of losses from these causes was often at hand.

The somewhat unusual shape of the curves for total basal area is perhaps the direct result of past losses from fire, insects, and disease. Under normal conditions the total basal area per acre might be expected to increase, although at a declining rate throughout the life of the stand. The material upon which this study is based shows no increase in total basal area after about 50 years of age. (Fig. 4.) This may perhaps be explained by the fact that many of the younger plots measured showed no evidence of fire, while practically all the older ones did. A normal increase in basal area may therefore be offset by decimation from fire and other agencies.

Attention should be called to another unusual result obtained in this study, namely, the reverse curvature in the intercept curves for total basal area and total volume in cubic feet. (Fig. 3.) Although studies with other species have generally yielded a simple convex curve for intercepts of total basal area and a straight line for intercepts of total cubic-foot volume, it did not seem possible with the data at hand to obtain a satisfactory check between the various curves without using reverse curvature as shown. To those who object to this unusual spacing of the final curves it may be pointed out that within the limits of the ordinary range of sites encountered in the region the peculiar form of the intercept curves is of no consequence. Very few localities are to be found where the site index is over 105 or less than 55.

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[10] REINEKE, L. H. Op. cit.

A check was obtained on the final curves for total cubic-foot yields, basal area per acre, average basal area, and total number of trees by comparing the actual values for each plot with values estimated from the curves for the given age and site index. The sum of the actual values for each factor was divided by the sum of the curve values to obtain an aggregate deviation. For average basal area the individual values were weighted by actual number of trees on each plot. Then the percentage deviation of the individual plots from the curve values were averaged without regard to sign to obtain the average deviation. The following results were obtained:

	Aggregate	Average deviation
Total cubic-foot volume per acre......per cent..	+3. 24	18. 42
Basal area per acre....................do....	−1. 90	17. 30
Average basal area....................do....	+. 33	19. 01
Total number of trees................do....	+1. 20	23. 41

From these figures it appears that in general the tables are conservative, since the actual plot values average higher than the curves. The comparatively high figures for average deviation give some measure of the great variation in stand character encountered even among plots selected for uniformity of stocking, but in application these wide variations are taken care of in part by making corrections for differences in stocking.

DETERMINATION OF THE SPRING-BROOD EMERGENCE OF ORIENTAL PEACH MOTHS AND CODLING MOTHS BY VARIOUS METHODS[1]

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INTRODUCTION

During the course of several years' investigations of the life history of the oriental peach moth, at Riverton, N. J., a number of interesting and important facts have been learned. Some of these pertain to the best methods of handling material. In this paper the writers present some of the results obtained from several methods used in determining the spring-brood emergence of the oriental peach moth (*Laspeyresia molesta* Busck) and of the codling moth (*Carpocapsa pomonella* L.). The purpose has been to develop a method which would give results that closely resemble the actual emergence of spring-brood moths in the orchard.

In this preliminary report only the more striking results are discussed. Lack of space prevents a detailed discussion of the relationship existing between the emergence and the weather conditions. The effect of temperature could be shown by graphs of daily temperature and emergence. All of the information relating to spring-brood emergence is shown in the tables and the plotted percentage curves. (Figs. 7, 8, and 9.) These curves show for each method the beginning and the end of the emergence period and also the dates when 2.5, 10, 25, 50, 75, 90, and 97.5 per cent (see arrows on curves) have emerged.

In 1924, when the senior author made his first detailed study on the life history of the oriental peach moth, and also in succeeding years, it was observed that spring-brood moths in an open screened insectary (shaded by a roof) emerge several weeks later than they normally do in an orchard. The normal orchard emergence was determined by means of bait pans and from the presence of fresh empty pupal skins. This information has shown that insectary material alone should not be relied upon for the beginning of a seasonal life-history study.

During the dormant season of 1925–26 some preliminary tests with various boxes and open screen cages containing overwintering oriental peach-moth larvae were made in a peach orchard. The cages and boxes were placed on the ground and 5 to 6 feet above the ground. Some of the cages faced south, others north, and still others faced upward. The differences in the emergence of the moths under the varying conditions were so striking in some instances that it was decided to make a more extensive study of this subject during the winter and spring of 1926–27 with oriental peach moths and codling moths.

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APPARATUS

During the dormant seasons of 1925–26 and 1926–27 several types of screen cages were employed. All types of cages were covered with 16-mesh copper screening, except type *B* which had 24-mesh galvanized screening.

In 1925–26 cages of types *A* and *B* were used. The type *A* cage measures 5 by 6 by 8 inches and is made of wood with 1-inch square wooden supports for the screening. About 75 cocoons may be tacked to the narrow detachable wooden strips (lathing) which are suspended on the inner surface of the solid back board. Type *A* cages were placed on poles or hung on the trunks of peach trees near the ground. (Fig. 1, A.)

The type *B* cage is similar to the type *A* cage but larger. (Fig. 1, B.) It measures 6 by 12 by 15 inches and contains four detachable wooden blocks (1 by 2.5 by 13 inches) on which approximately 300 cocoons may be placed. The solid wooden side of the cage is placed at the bottom, and the blocks rest on the inner and upper surface in such a manner that one end of each block is elevated slightly so rain will drain off readily. The cages were placed on the ground or on a rack 5 feet above ground and the cocoons in all cases faced upward.

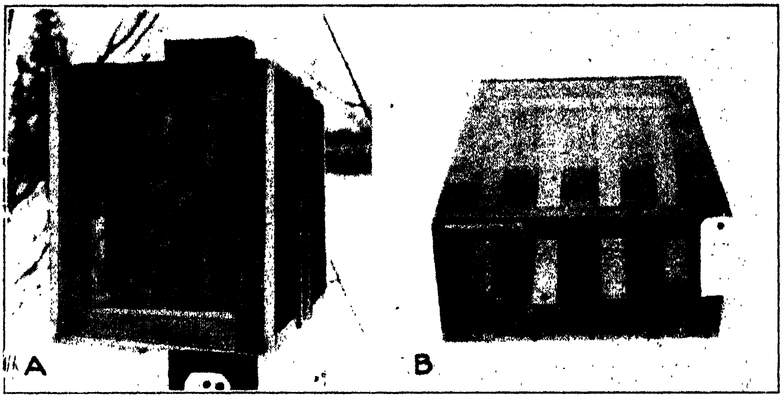

FIG. 1.—Screen cages used in spring-brood emergence studies of the oriental peach moth and the codling moth, Riverton, N. J., season of 1925–26; A, type *A* cage; B, type *B* cage

During the dormant season of 1926–27 cages of types *C* and *D* were employed. These cages are larger than those of type *A* and differ from both types *A* and *B* in that they have no wooden supports for the screening, consequently all shadows are eliminated except that thrown by the wire. The type *C* cage is 4 by 10 by 14 inches and opens like a book. (Fig. 2, A; fig. 3.) Cages of this type were used chiefly on poles with oriental peach-moth larvae. The type *D* cage is 6 by 9 by 13 inches, and the cocoons are placed on detachable blocks of wood (1 by 2.5 by 8 inches) which rest against the back of the cage. (Fig. 2, B.) These blocks may be removed through a 4 by 6 inch opening in the solid lower side. The opening is closed with a sliding metal plate. Cages of this type were used chiefly in 1927 for codling-moth larvae; they were used also in the moisture experiments in the insectary.

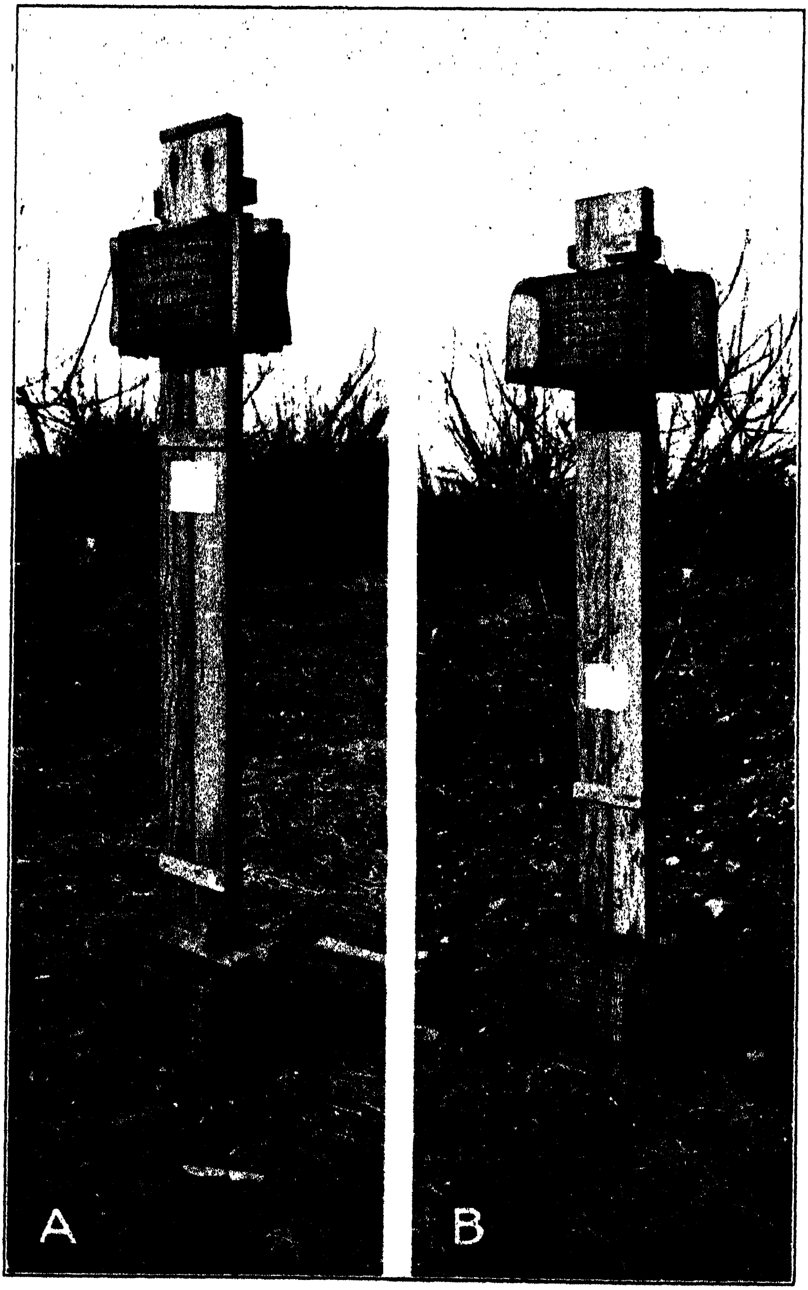

FIG. 2.—Screen cages on poles, used in spring-brood emergence studies, Riverton, N. J., season of 1926–27; A, type *C* cage; B, type *D* cage

The type *C* cage which opens like a book proved to be the most satisfactory to handle. An occasional adult may escape upon opening a cage of this type, but it is easily recaptured if the cage is opened before a window or on the light side of a screened insectary.

Type *E* cages (fig. 4, A) made of wire strainers of various sizes were also found useful when a small number of cocoons were under observation. These are easy to make and may be placed almost anywhere in an orchard. They were not used in any of the tests reported in this paper.

The covered wooden box (fig. 4, B; fig. 5, *a*) measures 6 by 13 by 16 inches, has a 16-mesh copper-screen bottom, round screen-covered holes on the sides, and a detachable lid covered with roofing paper. Two-dram homeopathic vials plugged with cotton, each containing one overwintering larva in a cocoon spun in a piece of corrugated paper, are placed within the box in an upright position in portable wooden frames. The capacity of the box approximates 300 vials. This box is very similar in structure to those used by T. J. Headlee in his codling-moth investigations, and the writers are indebted to him for the idea.

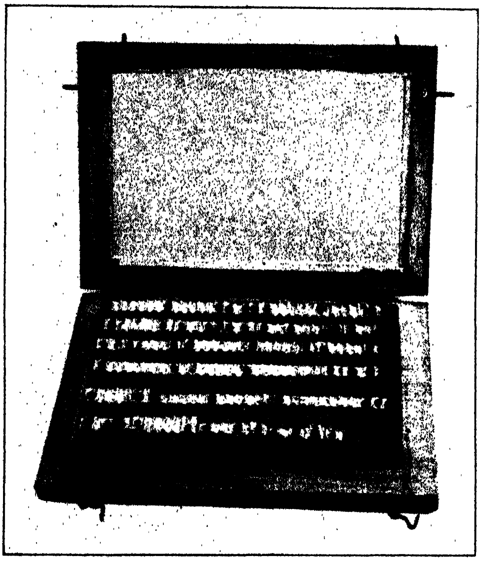
FIG. 3.—Type *C* screen cage, open

A heavy canvas band, about 5½ feet long, was so constructed that it held two parallel rows of 2-dram shell vials placed in small pockets. (Fig. 6.) There were approximately 220 vials in the two rows. These were plugged with cotton and placed in the pockets upside down so that water would not collect in them. The band was placed around the trunk of a large apple tree, approximately 2 feet above the ground. The past season (1926–27) overwintering larvae of the oriental peach moth in cocoons were placed in the canvas band. This canvas band resembles the canvas band used by W. A. Ross of Vineland, Ontario, to whom the writers are indebted for the idea.

The insectary, where a considerable quantity of overwintering material was kept, is an open room screened on three sides and covered with a hip roof covered with tarred roofing paper. In most cases the individual cocoons spun up in corrugated paper were placed in separate 2-dram vials which were plugged with cotton. The vials stood upright in wooden frames, which in turn were placed in wooden trays and kept in the center of the room about 4 feet above the ground. No direct sunlight reached the vials.

The packing house (or storage barn) used in the experiments was a medium-sized building (25 by 40 feet) located near Riverton, N. J., adjacent to a cherry orchard. The building had no windows, but had a large sliding door on the north side which was closed most of the time. The overwintering larvae spun up in corrugated paper were placed in a screen cage (11 by 21 by 21 inches), which was placed on the floor of the building, about 3 feet above ground, among the baskets (old and new) which were stored in the building.

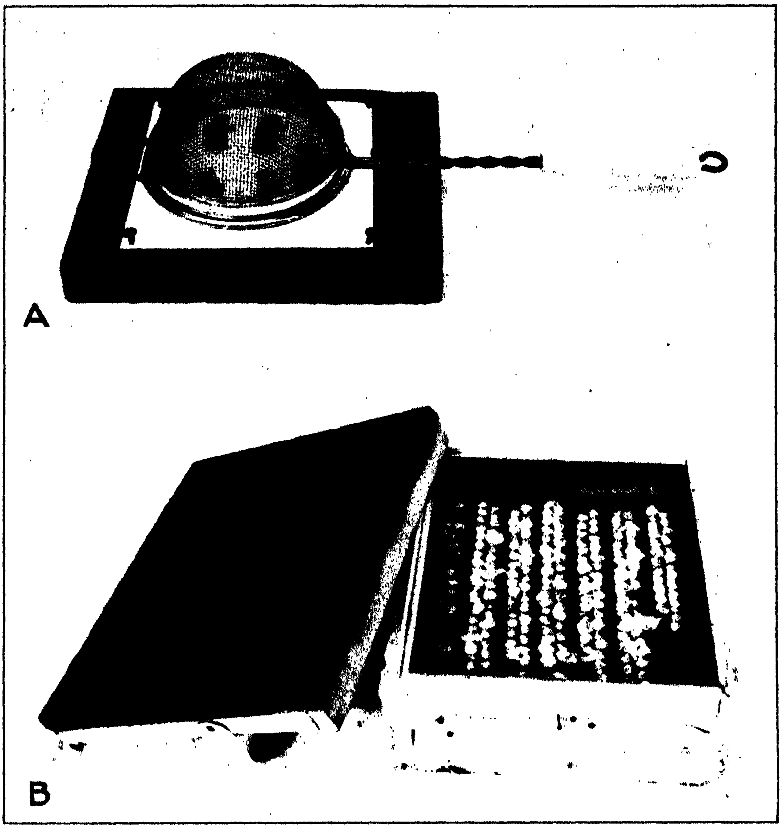

FIG. 4.—A, Type *E* screen cage, useful for a small number of cocoons; B, covered wooden box used in spring-brood emergence studies, Riverton, N. J.

METHODS

Most of the overwintering larvae, in cocoons, of the oriental peach moth and the codling moth used in the tests reported upon in this paper were spun up in corrugated straw paper. In a few instances the larvae in some of the boxes and a portion of the larvae in the canvas band spun their cocoons against the glass in shell vials. All pupation records were made by observing larvae that had spun their cocoons against the glass of 2-dram vials stoppered with cloth-covered cotton plugs.

Daily examinations were made from April 1 to midsummer of all material in the screen cages, boxes, canvas band, insectary, packing house, and elsewhere for adult emergence. Usually the moths of both species emerge during the morning hours. On warm days moths start to emerge at sunrise or shortly thereafter, whereas in cool weather emergence may not start until 9 o'clock or later and may continue until the early part of the afternoon. Counts and sex determinations were made each day late in the afternoon, including Sundays and holidays.

All overwintering cocoons of oriental peach moths were placed in their respective positions late in the fall or during midwinter, never any later than January. General observations and also two experiments show that the date of transferring the reared or collected overwintering larvae from the insectary to the outdoor cages or other containers has little or no influence on the emergence of the moths, provided the larvae are not kept in the insectary much after February 1. The date of placing material in the field seems to have a slight influence on mortality. Material placed outdoors in November or earlier may show a slightly greater mortality than similar material placed in the orchard during January.

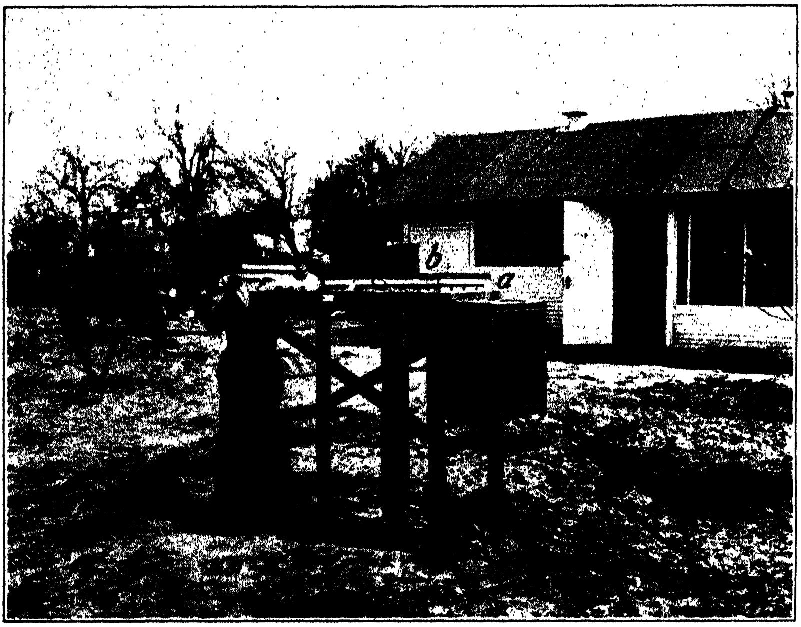

FIG. 5.—Wooden rack 5 feet high, supporting covered wooden boxes (*a*) and a type *B* screen cage (*b*). (Insectary in background)

All overwintering larvae of the codling moth used in the tests were collected from cloth bands in apple orchards during October and November. Whenever larvae were removed from their original cocoons on the trees they were placed in glass containers filled with corrugated paper strips and permitted to remain in a fairly warm room (temperature at least 50° F.) for 24 to 48 hours so they might spin new cocoons. This act of respinning cocoons probably has some influence on the mortality of codling-moth larvae.

TEMPERATURE

In conducting the studies reported in this paper, temperature records were kept for the several locations where overwintering larvae were placed, particularly during the season of 1926–27. A mass of data has been assembled on this subject; at this time, however, no attempt will be made to correlate these temperature data

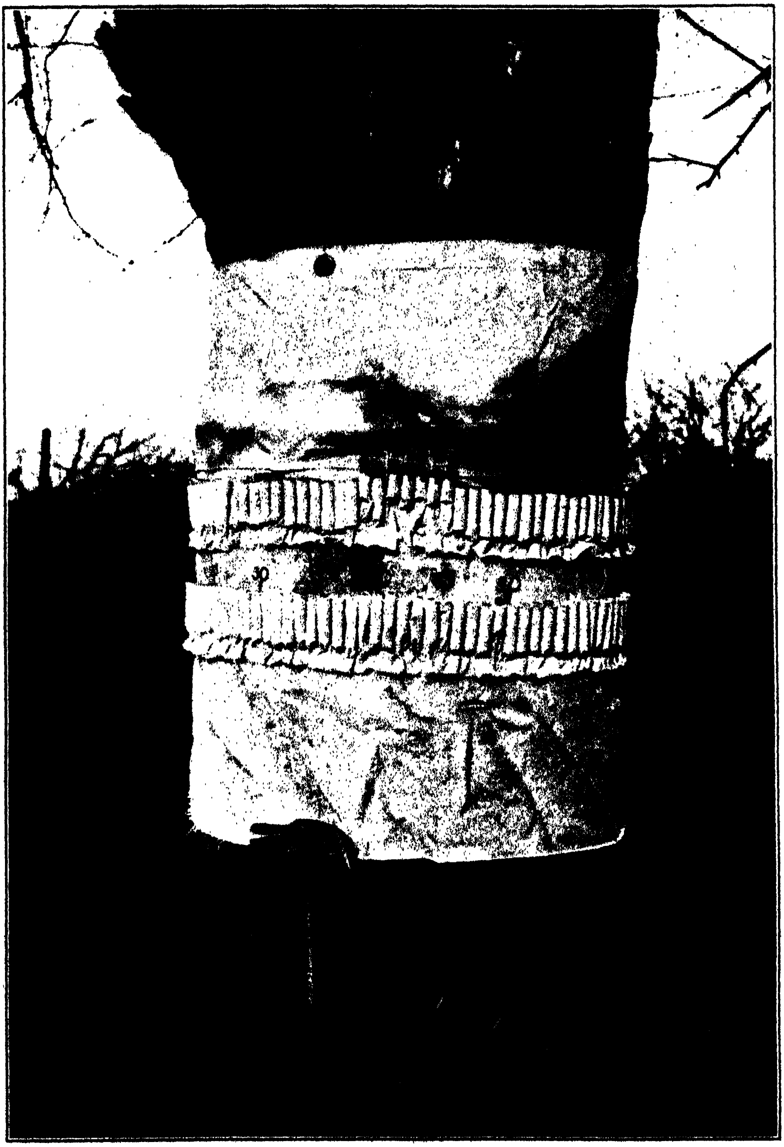

FIG. 6.—Canvas band, with flaps open, used in spring-brood emergence studies of the oriental peach moth, Riverton, N. J., season of 1926–27

in detail with the facts reported herein. The writers present this paper at this time in order to obtain constructive criticism which will be of assistance in future studies on temperature and other factors. Some of the more important facts learned to date from the temperature records will be discussed briefly.

The data show that there may be very great differences in temperature in the various situations in or about a fruit tree at any given time. This is particularly true on clear days when the sun shines brightly. In other words, the temperatures recorded on a thermograph placed in the shade in an orchard are not the actual temperatures to which many overwintering larvae are subjected, especially on clear days.

The decided differences in temperature that may occur on the north and south exposures of a fruit tree on clear days in the spring of the year (based on records in March, 1927) are shown in Table 1. It will be noted that the four spirit thermometers used in recording the temperatures were placed in four locations similar to those of the cages placed on the north and south sides of a pole. Midday readings on a clear day show temperatures on the south side that may be 15° to 20° or more above those on the north side, whereas on cloudy days all of the readings of the four thermometers may be the same. Also the temperatures near the ground are usually a few degrees higher than the temperatures 5 feet above ground with the same exposure.

TABLE 1.—*Comparison of temperature records from four spirit thermometers placed on the north and south sides of poles in locations similar to those of the cages, the thermometers facing north being shaded*

Date and conditions	Exposure	Height of bulb from ground	Temperatures at—									
			8 a. m.	9 a. m.	10 a. m.	11 a. m.	12 noon	1 p. m.	2 p. m.	3 p. m.	4 p. m.	5 p. m
			°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*
Mar. 10, 1927: Clear day; gentle wind	South	5 ft.	48.0	47.0	56.0	59.0	66.0	70.0	68.0	69.0	69.5	61.0
	North	5 ft.	37.0	38.0	44.0	46.0	50.0	53.0	54.5	55.0	54.5	52.0
	South	2 in.	48.0	48.0	58.0	64.0	70.0	74.0	75.5	74.0	69.0	59.0
	North	2 in.	38.0	39.5	46.0	50.0	53.0	56.0	58.0	57.0	56.5	53.0
Mar. 14, 1927: Cloudy day; gentle wind	South	5 ft.	60.0	60.0	63.0	62.0	58.0	57.0	57.5	57.0	56.5	57.0
	North	5 ft.	59.0	60.0	63.0	62.5	61.0	55.0	57.0	57.5	58.0	57.0
	South	2 in.	61.0	60.0	64.0	62.0	59.0	57.5	59.0	59.5	56.0	58.0
	North	2 in.	60.0	60.0	64.0	63.0	60.0	57.0	58.0	58.0	58.0	55.0

The information in Table 1 shows clearly that emergence data taken from a given lot of overwintering larvae placed in a box or some other container located in some one spot in an orchard, with all parts of the container subjected to the same temperature, will not coincide with the normal spring-brood emergence in the orchard. In choosing a method to determine spring-brood emergence, an investigator should take into consideration the decided temperature variations in sunlight and in shade to which overwintering larvae in an orchard are subjected. The two methods discussed in this paper, in which consideration is given to the effect of sunlight and shade on temperature, include the use of the open screen cages and the canvas band.

MOISTURE

Moisture seems to have some influence on the mortality and the development of spring-brood oriental peach moths and codling moths. Overwintering cocoons spun up in corrugated paper and kept dry under insectary conditions show a much greater mortality than cocoons soaked in water occasionally. (Table 3, M, N; Table 4, L, M.) During the 1926–27 season two cages (type *D*) of overwintering oriental peach-moth larvae and two cages of codling-moth material, each containing 300 or more larvae, were assembled in the fall and kept in an open screened insectary. From January 4 to July 5 the blocks of wood containing the cocoons of one cage of each species were removed once a week and submerged in water (at the temperature of the air) for two hours (the total time soaked being 54 hours) and then replaced in the respective cages. At each soaking the corrugated paper became thoroughly wet. In fact, in warm weather the soaking was so thorough that in a few instances it caused some of the larvae to abandon their cocoons.

These results showed that the wetted oriental peach-moth larvae produced adults a few days earlier than those which had never been in contact with water, while with the codling moth material the reverse was true in that the soaking delayed the emergence somewhat. This observation on the reaction of overwintering larvae of the codling moth to moisture does not agree with the results obtained and conclusions drawn by Townsend.[2] He soaked codling-moth cocoons several times at the rate of two hours per week, and the moths emerged sooner than from the dry material. The above wet and dry tests were repeated in 1927–28 with 1,000 or more individuals of each species. The emergence dates, particularly the peaks of emergence, for the wetted and dry material, coincided for each species. This corroborates the results obtained in previous tests and tends to support the conclusion that moisture has little or no influence on the time of emergence of the spring-brood moths.

MORTALITY OF LARVAE

The mortality (Tables 2, 3, and 4) of overwintering larvae of the oriental peach moth in all of the tests made was lower than that of the codling moth under similar conditions, except in one test (Table 3, N; Table 4, L). Although this may be a general and normal condition, the following facts probably had something to do with the results: All of the oriental peach-moth larvae used in the tests were reared in the insectary and therefore each larva spun but one cocoon. There was one exception to this; two cages (Table 2, F, G) contained field-collected larvae that spun their cocoons in corrugated paper, and the mortality in these open screen cages of type *B* was high (26 to 32 per cent) compared with the average (5.6 per cent) for reared larvae in open cages of type *C*. (Table 3, I.) As previously mentioned, all of the overwintering codling-moth larvae were collected from under burlap bands in October and November and respun their cocoons in corrugated paper. This respinning probably accounts for some of the mortality.

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[2] TOWNSEND, M. T. THE BREAKING-UP OF HIBERNATION IN THE CODLING MOTH LARVA. Ann. Ent. Soc. Amer. 19: 429–439. 1926.

The mortality of the overwintering material of both species in the cages of types *C* and *D* on the poles showed the lowest average (oriental peach moths 5.6 per cent and codling moths 15.2 per cent) for all of the methods used. (Table 3, I; Table 4, I.) The mortality of the material in the covered boxes (20 to 41 per cent) and also of the larvae in cocoons which were kept dry (28 to 45 per cent) in the insectary (not in vials) was high. Under insectary conditions the overwintering oriental peach-moth larvae in vials showed a comparatively low mortality (8 to 9 per cent).

The authors are of the opinion that an environment which shows the lowest mortality record is more likely to resemble a natural situation than an environment which produces a high mortality.

EMERGENCE OF MOTHS

EMERGENCE IN SCREEN CAGES

Field observations of the emergence of the spring brood of oriental peach moths have shown that overwintering larvae located on the south side of the trunks of fruit trees emerge much sooner than those located on the north side. It is evident that the direct rays of sun light striking the cocoons located on the south side raise the temperature considerably, and as a result the larvae on the south side undergo their changes or transformations much earlier than larvae under shaded conditions. With this information in mind the authors constructed open screen cages so the cocoons within could be fully exposed to sunlight.

Preliminary tests were made in the dormant season of 1925–26. Eight screen cages of type *A* (fig. 1, A) were placed on two poles in the midst of peach trees in a small peach orchard. Four of the cages faced north and four faced south. Two of the north cages and two of the south cages were next to the ground and two of the north cages and two of the south cages were 5 feet above the ground. Pole No. 1 was situated in an open space between the trees, and pole No. 2 was on the north side and adjacent to the trunk of a 4-year-old peach tree which had not been pruned for two years. The results obtained from the respective cages on the two poles were nearly alike, consequently they were combined, and the results are shown in Table 2, A–G, and Figure 7, A–G. However, the two south cages adjacent to the ground showed a small difference in that emergence in the south ground cage located on pole No. 2 was two days later than in the south ground cage located on pole No. 1. This difference was undoubtedly due to the fact that the trunk and larger branches of the tree south of pole No. 2 cast a shadow on the cocoons in the south ground cage.

The differences in the emergence from cocoons in type *A* cages on the south and north sides of a pole or tree and also adjacent to the ground and 5 feet above are shown in Figure 7, A–D. The cocoons on the south exposure produced adults much earlier than cocoons located in the shade. It was also noted that the cocoons near the ground produced adults 24 to 48 hours sooner than those 5 feet above ground on the same side of the pole or tree. These results agree in general with the differences in temperature noted in Table 1 for the four locations.

TABLE 2.—*Spring-brood emergence of oriental peach moths from overwintering larvae, kept under different conditions, Riverton, N. J., 1926*

	Container	Location and conditions	Number of cocoons	Number of moths	Larvae dead	Date of first emergence	Date of 50 per cent emergence	Time (days) required for emergence of— 10 per cent	25 per cent	50 per cent	75 per cent	90 per cent	100 per cent
					Per cent								
A	Cage type *A*	Pole, south, ground	[a]290±	273	[b]5.8	May 3	May 7	1	2	4	6	9	28
B	do	Pole, south, high	[a]140±	134	[b]4.2	do	May 8	2	3	5	7	9	24
C	do	Pole, north, ground	[a]255±	240	[b]5.8	May 9	May 17	3	4	8	15	20	34
D	do	Pole, north, high	[a]130±	124	[b]4.6	May 10	May 18	3	5	8	13	20	42
E	do	Four cages (A–D) combined.	[a]815±	771	[b]5.3	May 3	May 12	2	4	9	15	22	49
F	Cage type *B*	Rack, facing up, high.	247	167	32.3	May 2	May 6	2	3	4	6	8	23
G	do	Ground, facing up, under tree.	230	169	26.5	May 4	May 9	2	3	5	7	9	25
H	Covered box	Rack, vials, high	247	197	20.2	May 12	May 26	5	9	14	20	23	39
I	do	Ground, vials, under tree.	241	140	41.9	May 15	June 3	7	14	19	25	30	47
J		Insectary, vials, shaded.	1,087	981	9.7	May 14	do	12	16	20	27	31	55

[a] Number of cocoons approximated. [b] Based on approximate number of cocoons.

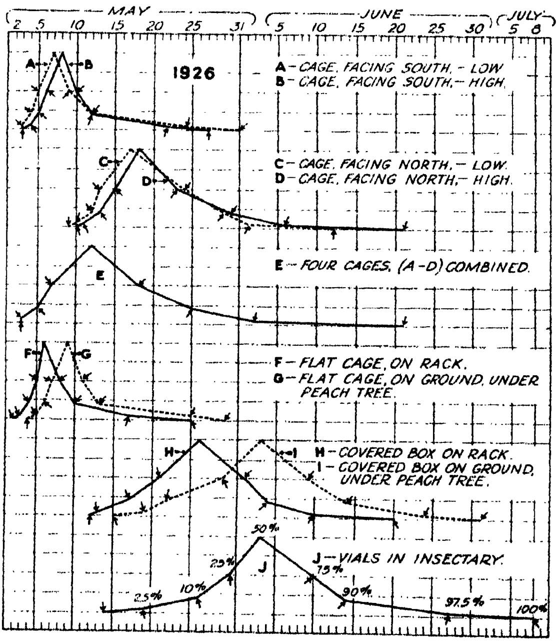

FIG. 7.—Emergence of the spring brood of the oriental peach moth, Riverton, N. J., 1926

In 1925–26 two lots of field-collected oriental peach-moth larvae were placed in two screen cages of type *B*. (Table 2, F, G; fig. 7, F, G.) One cage was located on a rack 5 feet high (fig. 5) and another was placed on the ground on the north side of a 4-year-old unpruned peach tree. The emergence in these cages was similar to that in the south cages on the poles. The chief difference was that the emergence in the cage on the ground was 48 hours later than in the cage on the rack. This seeming discrepancy may be attributed to the shadows cast by the branches of the peach tree on the cocoons in the ground cage.

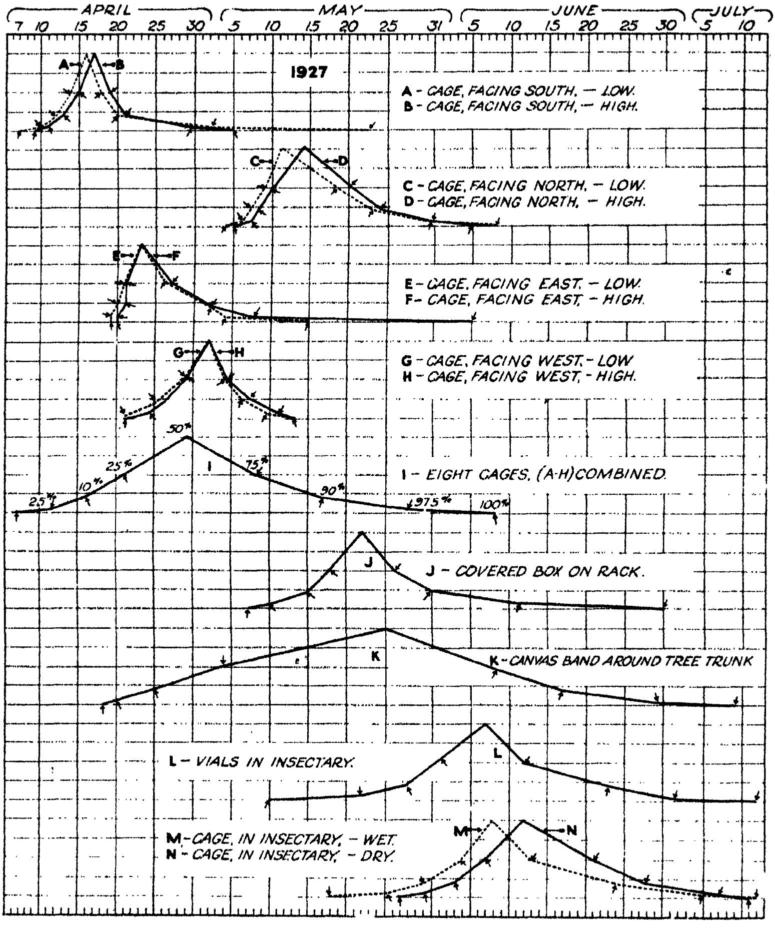

FIG. 8.—Emergence of the spring brood of the oriental peach moth, Riverton, N. J., 1927

More interesting and striking results with screen cages are shown for the dormant season of 1926–27. Eight screen cages of type *C* (fig. 2, A), each containing 300 to 400 cocoons, were placed on two poles in an open space in a small peach orchard. On pole No. 1 two cages faced south and two faced north, and on pole No. 2 two cages faced east and two faced west. On each pole two of the cages (one on each side) were next to the ground and two were 5 feet above the ground. Table 3, A–I, and Figure 8, A–I, show the results obtained in the respective cages.

TABLE 3.—*Spring-brood emergence of oriental peach moths from overwintering larvae kept under different conditions, Riverton, N. J., 1927*

	Container	Location and conditions	Number of cocoons	Number of moths	Larvae dead	Date of first emergence	Date of 50 per cent emergence	Time (days) required for emergence of—					
					Per cent			10 per cent	25 per cent	50 per cent	75 per cent	90 per cent	100 per cent
A	Cage type *C*	Pole, south, ground	331	301	9.0	Apr. 7	Apr. 16	5	7	9	11	13	46
B	do	Pole, south, high	314	287	8.5	Apr. 9	Apr. 17	4	6	8	10	12	26
C	do	Pole, north, ground	417	397	4.7	May 4	May 11	3	5	7	14	19	35
D	do	Pole, north, high	345	323	6.3	May 5	May 14	3	5	9	15	19	31
E	do	Pole, east, ground	353	345	2.2	Apr. 19	Apr. 23	1	2	4	7	13	25
F	do	Pole, east, high	331	320	3.3	Apr. 20	do	1	1	3	7	12	46
G	do	Pole, west, ground	271	248	8.4	Apr. 21	May 2	4	8	11	13	15	22
H	do	Pole, west, high	297	288	3.0	do	do	5	8	11	13	16	22
I	do	Eight cages (A–H) combined.	2,659	2,509	5.6	Apr. 7	Apr. 29	9	14	22	31	40	62
J	Covered box	Rack, vials, high	252	200	20.6	May 7	May 22	8	11	15	19	24	54
K	Canvas band	Vials about tree trunk.	326	285	12.5	Apr. 18	May 25	7	16	37	51	60	82
L		Insectary, vials, shaded.	1,698	1,559	8.1	May 10	June 7	18	22	28	33	44	63
M	Cage type *D*	Insectary, wet	304	255	16.1	May 18	June 8	12	17	21	26	37	55
N	do	Insectary, dry	342	186	45.6	May 27	June 12	7	11	16	25	32	45

The earliest emergence occurred in the south cages adjacent to the ground and the latest emergence occurred in the north cages 5 feet above the ground. There is a difference of 26 to 28 days in the peaks of emergence of the material in the south and north cages. This difference is considerably greater than in 1925–26 and may be attributed to the earlier spring and to the more open cages of type *C*, which did not have any wooden supports that threw shadows on the cocoons, as in type *A* or *B*. The emergence of the material in the east and west cages came between the periods of emergence in the north and south cages. There was a greater difference in the time of emergence in the east and west cages than expected. This may have been due to the fact that the cages (accidentally) did not face exactly east and west for a portion of the time (up to February 15) that they were in the field. The authors are also of the opinion that the apparent difference in the emergence dates was due in some measure to the fact that on numerous days in the spring before emergence started the sun shone brightly during the morning hours but was clouded in the afternoon. The cocoons in the east cages were therefore subjected to more hours of direct sunlight than those in the west cages, and consequently the accumulated temperature was greater. Tests similar to the above were repeated with codling moths and oriental peach moths in 1927–28 with the cages (type *C*) facing exactly east and west during the entire dormant period. The dates of emergence, particularly the peak of emergence, coincided exactly for the two species in the respective east and west cages.

The emergence results from the eight cages combined are shown in Figure 8, I. The authors are of the opinion that this curve closely resembles the curve of actual emergence of oriental peach moths in an orchard. This is probably true if one assumes that overwintering larvae are more or less equally distributed on all sides of tree trunks and branches and on the ground. If such is the case, it is probable that the total number of hours of direct sunlight received by the cocoons in the cages approximates the number of hours of direct sunlight received by overwintering larvae in the orchard. There is little or no published evidence to support or contradict this assumption.

In checking the emergence in the cages with the normal emergence in a peach orchard it was observed for three years that the appearance of the first adults or fresh pupal skins in the orchard on the south side of peach trees occurred on the same day or within 24 hours of the time when the first adults emerged in the open screen cages facing south and adjacent to the ground. Furthermore, the period of time when the greatest number of adults were captured in the bait pans coincides very closely with the peak of moth emergence in the eight cages. Bait pans are fairly satisfactory for determining the peak of emergence in an orchard, but they will not indicate accurately the date of the first emergence. Adults may be present in an orchard for 7 to 10 days before they come to baits. This is particularly true of oriental peach moths when an early spring emergence takes place.

The results obtained with overwintering codling-moth larvae in screen cages on poles are very similar to those obtained with oriental peach moths, except for the fact that the time of emergence is later, since it requires more degrees of effective temperature to make a codling moth emerge than an oriental peach moth. For both species of moths the position and nature of the curves which show the emergence in the various containers employed are similar. One of the differences noted is that there is a shorter period of time between the emergence in the south and north cages with the codling moth than with the oriental peach moth. This is probably due to the fact that the daily temperatures average much higher when codling moths are emerging than when oriental peach moths come out. The data relating to the emergence of the codling moth in screen cages, as presented in Figure 9, A–I, and in Table 4, A–I, are self explanatory and consequently will not be discussed.

TABLE 4.—*Spring-brood emergence of codling moths from overwintering larvae kept under different conditions, Riverton, N. J., 1927*

	Container	Location and conditions	Number of cocoons	Number of moths	Larvae dead	Date of first emergence	Date of 50 per cent emergence	Time (days) required for emergence of—					
								10 per cent	25 per cent	50 per cent	75 per cent	90 per cent	100 per cent
					Per cent								
A	Cage type *D*.	Pole, south, ground.	299	234	21.7	May 7	May 18	4	7	11	14	17	42
B	do	Pole, south, high	342	282	17.5	do	May 20	5	9	13	16	23	37
C	do	Pole, north, ground.	281	239	14.9	May 21	May 30	3	6	9	16	22	39
D	do	Pole, north, high	321	261	18.6	May 23	June 2	3	6	10	15	19	32
E	do	Pole, east, ground	303	283	6.6	May 10	May 19	3	6	9	12	18	38
F	do	Pole, east, high	310	265	14.5	May 13	May 22	4	7	9	14	20	36
G	do	Pole, west, ground.	278	233	16.1	May 11	do	5	7	11	16	22	30
H	do	Pole, west, high	342	301	11.9	do	May 23	6	9	12	18	26	41
I	do	8 cages (A–H) combined.	2,476	2,098	15.2	May 7	do	7	11	16	23	31	53
J	Covered box.	Rack, vials, high	519	385	25.8	May 19	June 7	10	13	19	22	25	36
K	Screen cage	Packing house	1,300	1,004	22.7	June 6	June 17	5	7	11	16	19	38
L	Cage type *D*.	Insectary, dry	325	234	28.0	May 28	June 10	6	10	13	15	25	48
M	do	Insectary, wet	315	259	17.7	May 29	June 12	5	10	14	19	23	48

EMERGENCE IN CANVAS BAND

A canvas band containing rows of vials filled with overwintering cocoons and placed about the trunk of a large fruit tree (fig. 6) is probably the next best method for determining the normal emergence of spring-brood oriental peach moths in an orchard. In a canvas band the larvae are evenly distributed about a tree trunk at a specified height. The vials are shaded by the canvas, yet the cocoons located on the sunny side of the tree are warmed by the direct rays of sunlight, consequently the moths emerge first on the south side and last on the north side.

If a comparison is made between the emergence in a canvas band (fig. 8, K, and Table 3, K) and in the eight open screen cages (fig. 8, I, and Table 3, I), it will be noted that the first moths appeared in the canvas band 11 days later than the first moths in the screen cages and the peak of emergence in the canvas band was 26 days later. Also the period of time required for all of the moths to emerge in the canvas band was 20 days longer than in the screen cages.

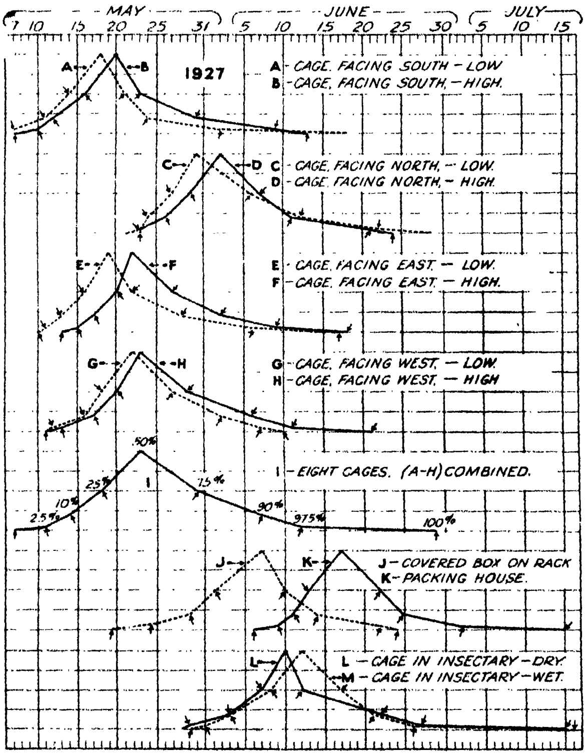

FIG. 9.—Emergence of the spring brood of the codling moth, Riverton, N. J., 1927

The authors are of the opinion that the following are objectionable points in the use of a canvas band for determining the normal emergence of adults in an orchard: In the first place, each cocoon is in a glass vial, which is by no means a normal condition. The vials collect and hold moisture and during the winter they may be filled with ice crystals. The canvas is apt to retain moisture for a longer time than would be normal on the tree trunk in dry weather. The thick cloth, the vial, and the corrugated paper provide a greater degree of protection and shade over the cocoons than that which normally occurs in most instances in the orchard. A single band at a given height on a tree trunk is a more restricted environment than is found in nature, where many larvae overwinter in cocoons on or near the ground or high up in the trees. The mortality of overwintering material in a canvas band is higher than in open screen cages; this indicates abnormal conditions.

No test was made with overwintering codling moths in a canvas band.

EMERGENCE IN COVERED BOXES

Covered wooden boxes (fig. 4, B) were used for two dormant seasons, 1925–26 and 1926–27. The data relating to emergence of oriental peach moths in these boxes are given for 1926 in Table 2, H, I, and Figure 7, H, I, and for 1927 in Table 3, J, and Figure 8, J. In the spring of 1926 the first oriental peach moths in the boxes emerged 9 to 12 days later than from similar material placed in screen cages (type *A*), whereas in 1927 there was a difference of 30 days in the dates of first emergence in the boxes and in the screen cages (type *C*). Also in 1926 the peaks of emergence of oriental peach moth material in the boxes were 14 to 22 days later than the peak of emergence of the combined south and north cages on the poles. Again, in 1927 the peak of emergence in the box material was 23 days later than in the eight screen cages (type *C*).

In 1925–26 the two boxes occupied different positions. One was on a wooden rack 5 feet above ground and exposed to the direct rays of sunlight, while the other was on the ground on the north side and adjacent to a 5-year-old peach tree which had not been pruned for two years. The material in the box on the rack emerged a few days earlier than the material in the box on the ground which was shaded by the tree. If the box on the ground had not been partially shaded it is probable that the moths would have emerged as soon as or earlier than the moths in the box on the rack. The temperature records show clearly that the ground temperatures are higher during midday than the temperatures 5 feet above ground, particularly on clear days, and the open screen cages show that material on the ground emerges sooner than up in the air if all other conditions are the same. If the boxes had been placed in a tree and partially shaded by the branches the emergence would undoubtedly have been as late as and probably later than that from the boxes placed on a rack and fully exposed to the sun all day.

A delay of two to three weeks in the emergence of oriental peach moths from overwintering larvae kept in a covered box has occurred for three seasons. The peak of emergence from the box material the past season (1926–27) was even later than the peak of emergence from cocoons facing north in the cages.

Overwintering codling-moth larvae were placed in covered boxes for two seasons. The results obtained for one season, 1926–27, are shown in Table 4, J, and Figure 9, J. In this test the first codling moths in the box emerged 12 days later than the first moth in the orchard or in the south cage near the ground. The peak of emergence in the box is also 15 days later than that in the eight open screen cages (fig. 9, I, and Table 4, I), which has been shown to resemble closely the normal emergence in an orchard.

In some respects the emergence from overwintering larvae kept in a box is comparable with the emergence occurring in a canvas band. Some of the objections raised concerning the use of a canvas band also apply to a covered box. In a covered box the overwintering larvae in corrugated paper are kept in glass vials plugged with cotton. The box serves as a partial insulation against rapid changes in temperature which occur in the spring of the year on clear days. It has been repeatedly noted that the temperature during midday within the boxes may be considerably lower than the outside air temperature. The mortality of overwintering oriental peach-moth larvae in covered boxes (20 to 41 per cent) is much higher than the average mortality (5.6 per cent) in the open screen cages. The foregoing facts indicate that overwintering larvae placed in a covered box behave differently from larvae in a more natural environment.

EMERGENCE IN INSECTARY

For several dormant seasons overwintering oriental peach-moth larvae have been kept in a screened insectary. Each year the beginning date and the peak-of-emergence date have been several weeks later than the normal emergence in the orchard or in the open screen cages. The greatest difference occurred the past season, 1926–27. The first emergence in the insectary, May 10, occurred 33 days after the first emergence in the orchard or in the screen cages, April 7, and the peak of emergence in the insectary, June 8, was 40 days later than the peak of emergence, April 29, in the eight cages on the poles. Also the emergence period in the insectary was approximately as long as the emergence period in the eight screen cages. Data for 1926 are shown in Figure 7, J, and Table 2, J, and for 1927 in Figure 8, L–N, and Table 3, L–N.

Codling-moth larvae kept in an insectary also emerge two to three weeks later than the normal emergence in an orchard. (Table 4, L, M, and fig. 9, L, M.)

The striking differences in the dates of emergence of insectary material and of normal outdoor larvae are of decided importance if a life-history study under insectary conditions is contemplated or if control recommendations are to be made which are based on spring-brood emergence of moths in an insectary.

EMERGENCE IN PACKING HOUSE

During the season of 1926–27 the writers did not have sufficient oriental peach-moth material to place a goodly number in a grower's packing house. Previous experience has shown that the emergence of oriental peach moths in an open screened insectary closely resembles the emergence that occurs in an open packing house where a grower may store old baskets that contain overwintering larvae.

In 1926–27 overwintering codling-moth larvae were placed in a closed packing house among old and new fruit baskets. From this material moths emerged much later than from larvae in the orchard. (Table 4, K, and fig. 9, K.) The first adults in the packing house, June 6, appeared 30 days later than the first adults in the orchard, May 7, and the peak of emergence in the packing house, June 17, was 25 days later than the peak of emergence in the open screen cages, May 23.

This decided delay in the emergence of moths in a packing house is important, especially if the packing house is located near a block of fruit trees, where adults which emerge late are likely to interfere with a control program based upon the normal emergence of moths in the orchard.

SUMMARY AND CONCLUSIONS

It has been repeatedly observed in orchards that overwintering larvae of the oriental peach moth and the codling moth located on the southern exposure of tree trunks and usually near the ground are the first individuals to change to pupae and emerge as moths in the spring, while moths that emerge late usually come from cocoons located in the most shaded portions of a tree or orchard.

There is a marked difference in the temperature on the south and north sides of a fruit tree on clear days in the spring, whereas little or no difference occurs on cloudy days. Apparently differences in temperature on a sunny exposure and the air temperatures in the shade are directly proportional to the amount of sunlight present.

The presence or absence of moisture about overwintering larvae seems to have a comparatively small influence on the emergence period of the moths; however, it does influence mortality. Overwintering larvae spun up in cocoons in corrugated paper kept in a dry insectary suffer a much greater mortality than similar material wetted frequently. In the various methods employed to determine emergence, the lowest mortality occurred in material confined in open screen cages located on poles or tree trunks where rain, dew, and snow could reach them.

Of the methods tested by the authors, the most satisfactory for determining the normal emergence of spring-brood moths in an orchard proved to be that involving the use of eight screen cages placed on poles (or tree trunks), so that four of the cages were adjacent to the ground and four were 5 feet above ground, and one cage of each group of four faced north, east, south, and west. This method subjects overwintering larvae to all of the elements, rain, snow, dew, wind, and sunshine. It takes into consideration the differences in temperature near the ground and several feet in the air, and also the decided differences in temperature which occur on sunny and shaded exposures. This method permits an equal distribution of larvae in all possible situations which, so far as known, are similar to those in nature. Since there is no evidence to the contrary, it is assumed that overwintering cocoons of both species are more or less equally distributed on all sides of the large branches and trunks of fruit trees and also to some extent on the ground. If such is the case, it is possible that the total hours of direct sunlight received by cocoons in an orchard would approximate the total hours of direct sunlight received by the cocoons located in the eight open screen cages placed as described above.

For two or three seasons the beginning date and the peak-of-emergence date for both species of moths in the screen cages compare very favorably with similar dates in the orchards.

The moth-emergence dates for the respective species of overwintering larvae kept in a canvas band, in covered boxes, in a screened insectary, and in a closed packing house are later or, in some instances, very much later than the normal emergence dates in an orchard or in the screen cages. The latest emergence for oriental peach moths occurred in the insectary, whereas for codling moths the latest emergence took place in a packing house.

A WILT DISEASE OF ALFALFA CAUSED BY FUSARIUM OXYSPORUM VAR. MEDICAGINIS, N. VAR.[1]

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INTRODUCTION

It is the purpose of this paper to report in detail the results of the investigations of the Fusarium-wilt disease of alfalfa (*Medicago sativa* L.) that recently was described briefly by the author (*21*).[3]

REVIEW OF LITERATURE

As stated in the above-mentioned paper (*21*), several writers have reported a root rot or wilt of alfalfa with which a species of Fusarium has been associated. Cottam (*3*) states that the pathogenicity of a root disease of alfalfa in Utah has been traced to the activity of a Fusarium. He describes this disease as being characterized by brown cankerous depressions at the bases of lateral roots, which are entirely destroyed, and he further states that the pathogene attacks both the parenchymatous tissue and the xylem vessels of the taproot. According to Cottam's observations, the diseased plants serve as centers from which the infection spreads in various directions, often destroying an entire field in a single season. He gives no description of the organism and cites no experiments to prove its pathogenicity. Judging from his description, the disease is evidently not the one under consideration in this paper.

Arnaud (*1*) describes a disease of alfalfa in France that he attributes to *Neocosmospora vasinfecta* (Atk.) Smith. He emphasizes the presence of the brick-red perithecia on the diseased roots, and apparently concludes that they belong to the fungus that causes the disease. He cites no proof that the disease under observation was caused by a Fusarium, although he considers it to be the same disease as that occurring on cotton. He compares the disease to that caused by Rhizoctonia and does not mention any symptoms that would indicate that he was dealing with a wilt.

McCallum (*7*) discusses a root rot attributed to a Fusarium that attacks alfalfa, tomatoes, melons, cantaloupes, and other crop plants, and states (*8*) that the most destructive diseases as a group in Arizona are those due to Fusaria. To this group belong the root rots or wilts of alfalfa. These diseases on alfalfa are said to be very destructive, often ruining a large part of the crop. Affected areas in the field

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[2] The writer is indebted to H. L. Westover, senior agronomist, and T. F. Akers, agent, of the U. S. Department of Agriculture, for sending much of the field material studied and for assistance with the experimental and survey work at West Point, Miss.

[3] Reference is made by number (italic) to "Literature cited," p. 432.

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usually start as small spots that gradually enlarge in a more or less concentric manner. The first indication of the disease is shown by a yellowing of the outer leaves of the affected plants. This chlorotic condition gradually becomes more pronounced until all the leaves and even the stems have lost their green color. This is either accompanied, or more usually followed, by the wilting and death of these parts. McCallum gives no description of the fungus. From his meager accounts it is impossible to tell whether or not he is describing the disease that the present writer has studied. Judging from his description of the manner in which the disease spreads in the field, it is thought that the two diseases are different.

Brown and Gibson (*2*) give a photograph of an alfalfa plant affected with a crown rot and discuss a root rot caused by *Fusarium* sp. Apparently they did not isolate the fungus and prove that the disease in question was due to the Fusarium associated with the lesions.

Selby (*15*), Selby and Manns (*16*), Piper (*10*), Weniger (*22*), and others discuss a root rot of alfalfa attributed to *Fusarium roseum*.

Tehon (*18*, *19*) reports root and crown rot and wilt of alfalfa from Illinois. A personal conference has developed the fact that these diseases differ from the wilt disease described in the present paper.

Robbins and Scott (*12*) report a Fusarium species closely associated with a blighting of alfalfa. Scott (*14*) states that in studies of Fusarium blight of alfalfa additional species of the fungus were isolated, but apparently none that had been previously reported.

Weimer (*20*) recently has described various types of alfalfa root diseases from many of which a species of Fusarium could almost always be isolated. However, the disease could not be reproduced by inoculating these Fusaria into healthy plants. When conditions were made extremely favorable for the fungi, damping off of seedlings could be produced with most of the organisms isolated.

Reports of alfalfa root rots or wilts have been received by the Plant Disease Survey from nearly every State where alfalfa is grown. These reports show that Fusaria are commonly associated with alfalfa-root troubles in many States.

Still further references could be given in which workers have found a species of Fusarium associated with a type of root rot or blight of alfalfa. However, in no case has the writer found a description of a typical Fusarium wilt such as is common in many other crops. The Fusarium wilt in other hosts is so well known that this disease in alfalfa would undoubtedly have been described in a manner that would leave no doubt as to its nature had it been seen by the other workers who have reported root diseases of this plant.

THE DISEASE

DISTRIBUTION AND ECONOMIC IMPORTANCE

The Fusarium wilt of alfalfa was first found in September, 1926, by H. L. Westover at the experiment farm near West Point, Miss., where forage-crop field experiments were conducted in cooperation between the Bureau of Plant Industry and the State Agricultural Experiment Station, and specimens were submitted by him to the writer for diagnosis. Later a survey was made of the alfalfa-growing sections of northeastern and east-central Mississippi and of central Alabama. The disease was found in several fields in northeastern

Mississippi near Aberdeen, Muldon, and West Point, but not in Alabama.

In April, 1926, B. A. Madson sent the writer specimens of diseased alfalfa plants from Chino, Calif. Among these were two plants that exhibited all the symptoms of Fusarium wilt. From these plants a Fusarium that appeared to be identical with that from Mississippi was isolated. Inoculation experiments were made and infection obtained. The Fusarium was reisolated and again used in inoculation experiments with positive results. Later, however, one of the check plants became infected. As this made the earlier results of questionable value, the experiment was repeated, a culture of the fungus from the original isolation being used. This test gave negative results under conditions similar to those in which the Fusarium from Mississippi gave a high percentage of infection. There is therefore some doubt as to the occurrence of this disease in southern California. It is possible that a mixed culture was obtained from the original isolation and that the parasite was overgrown by a saprophyte and eventually lost.

Although the occurrence of the disease has been definitely proved only in Mississippi, it seems probable that it will eventually be found elsewhere. From the present knowledge of the disease no reason can be seen why its distribution should be so limited. The disease appears to be too limited in its distribution to be of very great economic importance at the present time. In fields where it does occur, however, it causes the stand to thin out gradually. From a plot of 1-year-old alfalfa growing on the Government experiment farm at West Point, Miss., plants from areas about a yard square were dug up and the percentage of diseased plants was determined. Three areas were dug to get a representative sample from different parts of the plot. The plants were cut open and examined carefully for vascular browning. Counts made in this manner in April and August, 1927, showed an average of 12.5 and 8.5 per cent, respectively, of infected plants. The fields in which the disease has been seen had from a trace to 15 per cent of affected plants as nearly as could be estimated.

SYMPTOMS

The symptoms of this disease vary. Sometimes the upper leaves turn bright yellow, this color gradually fading to a light buff and finally to the color of dead and bleached alfalfa straw. Again, some of the basal leaves may have a tinge of old rose or some shade of pink. In other cases the tips of the stems droop and wilt during the warm part of the day or when the soil becomes too dry, but they may become turgid again when more water becomes available or transpiration is decreased. Sometimes the leaves die rapidly and become dry and brittle, but they retain much of their green color just as they do when cut for hay. Usually the disease becomes evident first in one stem that may be entirely dead before any signs of infection appear elsewhere. The entire plant dies gradually, sometimes requiring weeks or even months to succumb entirely. Stunting of the plant is sometimes an early symptom, but often this is not noticed until the plant shows evidence of approaching death. The plant at the left in Figure 1 shows an extreme case of dwarfing. Probably the other plants were infected at the time the photograph was taken, as they showed evidence of a diseased condition soon afterwards, but at that time they were as large as the controls. In Figure 2 the plants at the right show different stages of the disease, and those at the left are uninoculated controls.

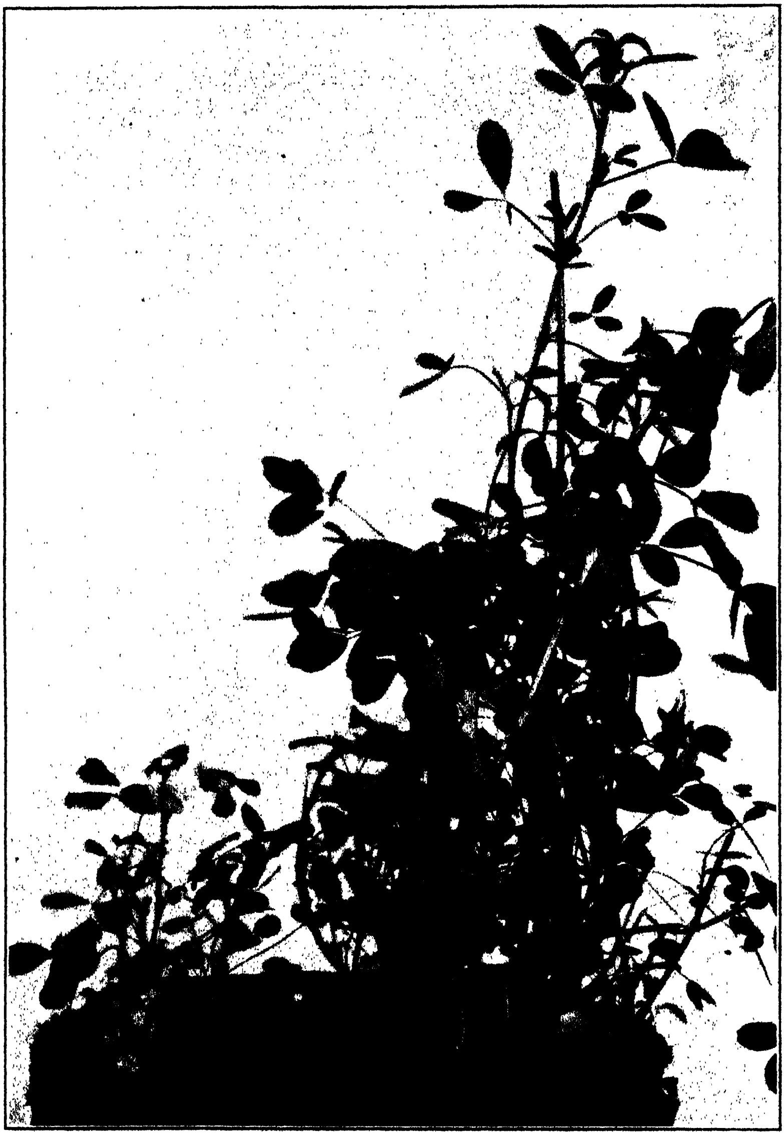


FIG. 1.—The dwarfing effect of the Fusarium-wilt disease of alfalfa is shown in the alfalfa plant at the left, which was inoculated by inserting spores and hyphae beneath the bark of the taproot near the crown. The plants at the right were inoculated also, but infection did not become evident until later. About one-half natural size

The roots of affected plants usually appear normal in the early stages of infection. Sometimes the roots of young diseased alfalfa plants are darker in color than normal because of the browning of the vascular bundles within, resembling in such cases young sweet-potato (*Ipomoea batatas*) plants affected with the so-called blue-stem disease caused by *Fusarium hyperoxysporum* Wollenw. As in other diseases of this type, the infection becomes evident when the vascular region is exposed by the removal of the outer bark of the root. The color of the invaded vascular system varies considerably, but usually ranges from cinnamon brown to mummy brown as given in Ridgway's (*11*) color standards. The amount of discoloration

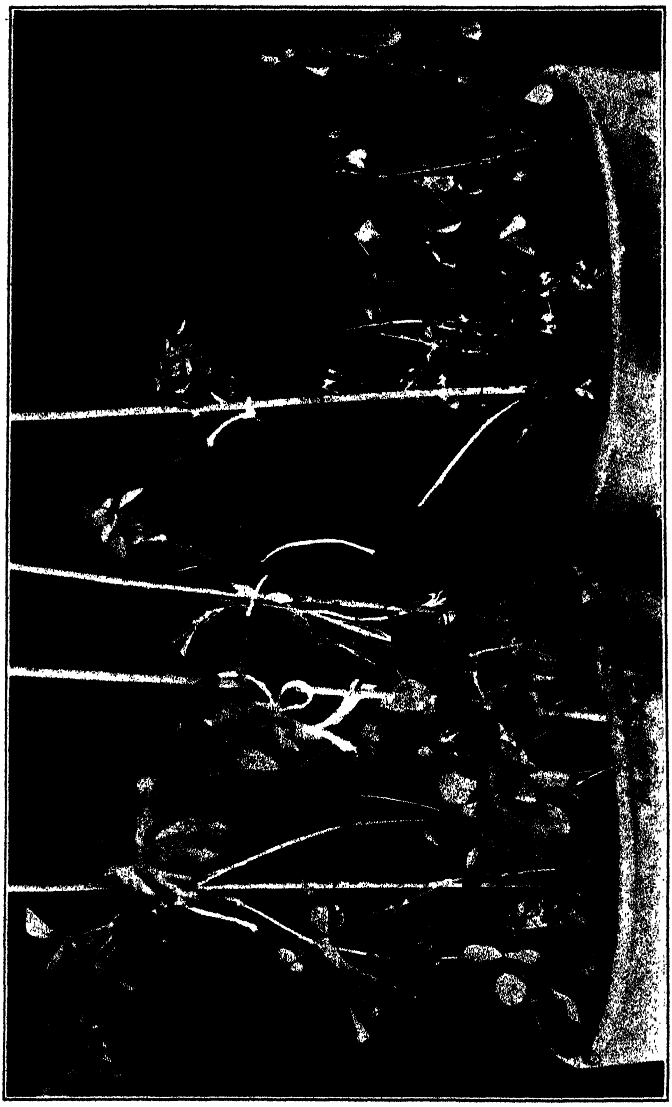


FIG. 2.—The alfalfa plants at the right (B) show various stages of the Fusarium-wilt disease; those at the left (A) were uninoculated controls. About three-eighths natural size

varies greatly, depending upon the extent to which the infection has spread. When only one stem or a part of the top is killed, a corresponding part of the vascular system of the root is involved. This is illustrated in the cross section of the root in Figure 3, A, in

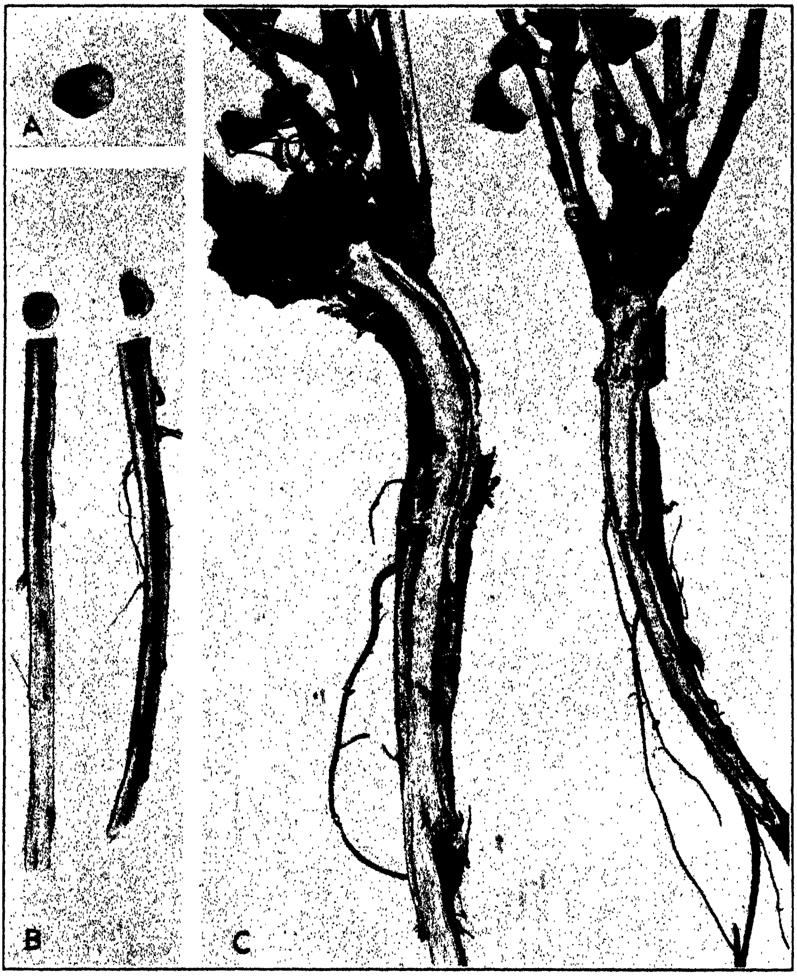


FIG. 3.—A, cross section of alfalfa root affected with Fusarium wilt, showing only one side of the vascular ring darkened. ×2½. B, cross and longitudinal views of taproots of two young alfalfa plants inoculated with *Fusarium oxysporum* var. *medicaginis*. Only a part of the top of the plant at the left was dead, owing to the fact that the fungus had invaded only one side of the root, as illustrated by the darkened vascular region on the left side of the cross section. The disease was more advanced in the root at the right. The top was nearly dead; the vascular region was entirely darkened, as shown in the cross section; and the disease had progressed farther downward, as illustrated in the longitudinal view. ×2. C, two alfalfa plants infected in the field at West Point, Miss. The darkened region just beneath the bark shows the area invaded by the fungus. ×2½.

which the vascular ring at the top is discolored. In advanced stages of the disease a large part of the vascular region of both the root and the lower part of the stem may be affected. Figure 3 shows the darkened vascular systems of artificially inoculated (A and B), and naturally infected (C) plants.

Anyone familiar with the Fusarium wilts of other plants should be able to recognize this disease of alfalfa without the slightest difficulty. The only other disease of this plant known to the writer that might be confused with it is the bacterial wilt caused by *Aplanobacter insidiosum* L. McC., recently described by Jones (*5*) and Jones and McCulloch (*6*). One of the most common symptoms of this bacterial disease is a dwarfing of the plant. A very typical example is portrayed in Plate 1, A, of the paper by Jones and McCulloch (*6*). The stems are slender, the leaves are small, and the dwarfed plants are usually paler than their healthy neighbors. No such dwarfing has ever been seen in field plants affected with Fusarium wilt. When the bark of the roots of plants affected with either of these diseases is split open, the vascular regions thus exposed are found to be discolored. This color is yellow or light brown in plants affected with the bacterial wilt and cinnamon to mummy brown in case of the Fusarium-wilt disease.

ETIOLOGY

The Fusarium wilt of alfalfa is caused by the fungus *Fusarium oxysporum* var. *medicaginis*, n. var.

SOURCE

As stated previously, the first diseased plants were sent from West Point, Miss., by H. L. Westover. The fungus was readily isolated by planting some of the diseased tissue on acid agar. Single-spore isolations made from the original culture were used for all of the studies herein described. However, isolations made from many other plants obtained at later dates were used in comparative studies. The fungus was isolated from the stems as well as from the roots of diseased plants.

MORPHOLOGY

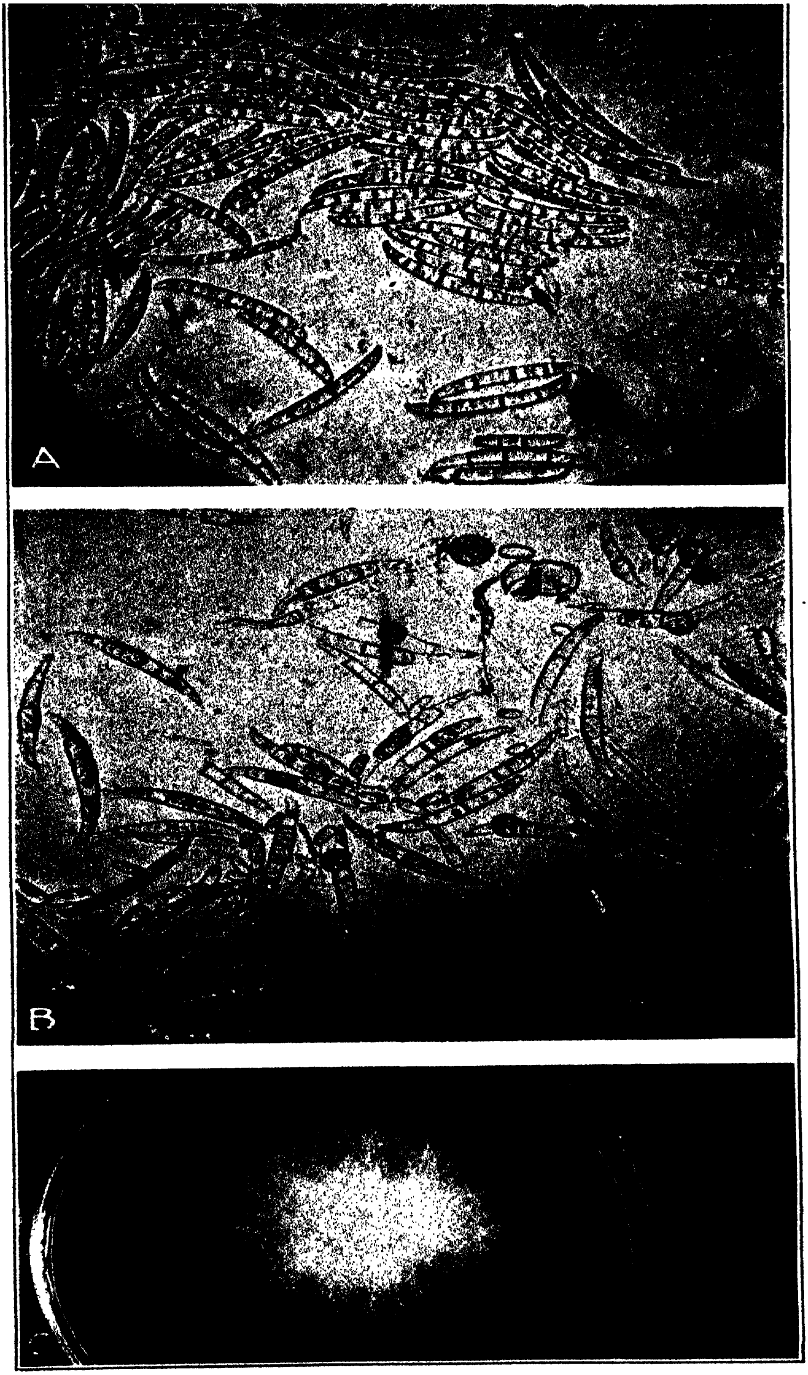
The morphological characters are as follows: Microconidia abundant, ellipsoidal to cylindrical, 0-septate and 1-septate. Macroconidia gradually attenuate toward both ends, usually nearly cylindrical in the middle half of their length, typically not broader toward the apex, usually somewhat pedicellate; 3-septate, dominant; average 40 by 4.4 μ and range 25 to 50 by 4 to 5.5 μ; in sporodochia and pseudopionnotes; 4-septate macroconidia common; 5-septate frequently seen; 1-septate, 2-septate, and 6-septate rarely present in sporodochia; in mass usually pale ochraceous buff to pale ochraceous salmon; sporodochia small to medium (up to 2 mm. in diameter), often converging into pseudopionnotes; chlamydospores terminal and intercalary, borne singly or in short chains, also commonly present in spores, in mycelium measuring 7 to 11 μ; aerial mycelium typically moderately well developed usually not over 5 mm. high, mostly white. Sclerotia on steamed potato tuber at first light buff to pale flesh, later light blue green becoming bluish black with age. The characteristic spore types are illustrated in Plate 1, A and B.

CULTURAL CHARACTERS

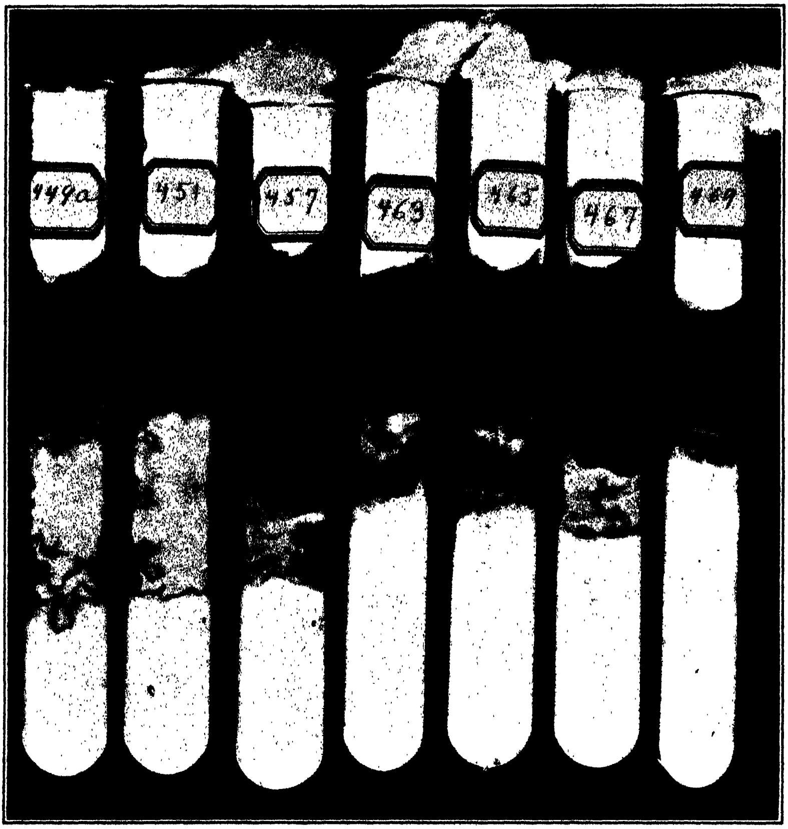
In the course of these investigations *Fusarium oxysporum* var. *medicaginis* was grown on several of the culture media commonly used for Fusaria. The medium used most and on which the fungus sporulated most vigorously was nutrient agar prepared from the dehydrated bactonutrient agar plus 2 per cent of dextrose. Additional sugar inhibited sporulation and stimulated color production. The typical growth on this medium after four days at 25° C. is illustrated in Plate 1, C. In tube cultures of this medium containing 2 per cent dextrose the mycelium typically was moderately well developed, usually not over 5 mm. high, mostly white except for tinges of light purple when in contact with the medium, and sporodochia and pseudopionnotes were formed in abundance. When 5 per cent or more of dextrose was added a faint orange-pink color developed in the aerial mycelium. The color varied from green-blue slate to slate with occasional tinges of some shade of light purple in the mycelium in contact with the medium. Chlamydospores are formed abundantly in both the spores and the mycelium in cultures on this medium, and sometimes small sclerotia are also formed. Steamed rice in test tubes was probably the most helpful medium in distinguishing this fungus from others that were isolated from time to time from roots with secondary infections. Not only could many secondary Fusaria be eliminated on this medium, but also the alfalfa-wilt Fusarium could be differentiated from other species that were being studied and that were known to cause wilt in other hosts.

The color produced by the alfalfa-wilt Fusarium on rice steamed one hour a day for three days varied from coral pink to Eugenia red to carmine red (*11*). A tinge of color varying from magenta to dull dark purple was present at times where the hyphae came in contact with the tubes. There was usually a small amount of warm-buff color in the rice beneath the hyphae. In a comparative study of this and other Fusarium-wilt fungi the outstanding difference was found to be the rate at which the alfalfa Fusarium penetrated the medium. Comparative tests were made at different times, always using rice tubes prepared in the same manner and handled as nearly as possible in the same way. Cultures of Fusaria from cotton, watermelon, cowpea, tomato, sweet potato, potato, and cabbage were furnished by various workers in the United States. In some cases two or more strains of an organism were obtained from different sources. With the exception of the Fusarium from cabbage, which produced no color, there was nothing strikingly different in the color reactions of these fungi on rice. In practically every case, however, the Fusarium from alfalfa could be separated from the others by the rate at which its mycelium penetrated the rice. The other species formed only a mycelial mat on the surface, while the Fusarium from alfalfa penetrated the medium, forming pockets that were lined with white mycelium. This difference is illustrated in Plate 2, where the two tubes at the left (449a and 451) are the alfalfa Fusarium, while the others are (left to right) from sweet potato, cowpea, watermelon, cotton, and tomato, in the order named. While this character probably will not always hold, it has remained fairly constant in the many tests made in these studies. The alfalfa-wilt Fusarium has more white aerial mycelium and more pink and less purple color in the mycelial mats than is present in those from other hosts. These are only slight physiological differences that may be helpful, but they are not considered to have any specific value.

As *Fusarium oxysporum* var. *medicaginis* formed no sporodochia on potato-tuber plugs, no spore measurements on this medium are given



A, Photomicrograph of macroconidia and microconidia of *Fusarium oxysporum* var. *medicaginis*. The spores were from a pseudopionnotes in a 7-weeks-old culture on nutrient agar. × about 500. B, Photomicrograph of macroconidia, microconidia, and chlamydospores from a 3-months-old culture of *F. oxysporum* var. *medicaginis* on potato agar. × about 500. C, A typical 4-day-old colony of *F. oxysporum* var. *medicaginis* growing on nutrient agar at 25° C. Slightly reduced.



Rice cultures of six different Fusarium wilt fungi 27 days old isolated from alfalfa (449a and 451), sweet potato (457), cowpea (463), watermelon (465), cotton (467), and tomato (469). These cultures are of the same age and are on the same medium, made by transferring hyphae from Petri-dish cultures also of the same age and on the same medium. Note the greater rapidity with which the alfalfa fungus penetrates the medium and forms pockets lined with white mycelium. The growth of the other fungi was limited largely to the formation of a mat of mycelium on the surface of the medium below. A white, rather compact mycelial growth formed over the surface of the plugs, with an occasional blue sclerotium, but no other color was produced. On potato agar without sugar the mycelial growth was rather sparse and no color was produced, but thin pseudopionnotes were frequently formed. When 5 per cent dextrose was added a faint orange-pink tint was present in the mycelium in parts of the culture, while scattered about were patches of color varying from green-blue slate to slate. This fungus does not grow very luxuriantly on oat, corn-meal, or Lima-bean hard agars made according to Sherbakoff's directions (*17*). Small sporodochia were formed on most of the cultures, and a small amount of color varying from Rood's violet to raisin purple was present in all except those on Lima-bean agar, where no color was developed. A fairly luxuriant growth of white aerial mycelium and some sporodochia were produced on *Melilotus alba* stems.

The spore measurements were made under the oil-immersion lens. At least 25 and usually more spores (but fewer in case of the 1, 2, 5, and 6 septate spores) from sporodochia or pseudopionnotes, were used for each set of measurements. The dimensions of the spores on the various media are as follows:

- On 2 per cent nutrient agar plus 2 per cent dextrose; culture 3 months old:
  - Macroconidia—
    - 2-septate.—37 by 4 μ. Rare.
    - 3-septate.—36.2 by 4.58 (25 to 47 by 4 to 5.4) μ. Up to 95 per cent.
    - 4-septate.—44.1 by 4.89 (41.4 to 48.6 by 4.5 to 4.95) μ. Up to 15 per cent.
    - 5-septate.—37.8 by 5.17 (34.2 to 41.4 by 4.95 to 5.4) μ. Only 2 spores measured; up to 1 per cent.
    - 6-septate.—48.6 by 5.4 μ. Only 1 spore measured; rare.
  - Microconidia—
    - 0-septate.—7.88 by 3.75 (4.5 to 12.6 by 2.7 to 5.4) μ.
    - 1-septate.—11.34 by 3.91 (9 to 12.6 by 3.6 to 4.5) μ.
  - Chlamydospores—
    - In spores.—Intercalary, 8.5 by 7.46 (7.2 to 10.8 by 5.4 to 9) μ.
    - In mycelium.—(a) Intercalary, 9.9 by 9.2 (7.2 to 10.8 by 7.2 to 10.8) μ; and (b) terminal, 11.3 by 11.1 (10.8 to 12.6 by 10.8 to 12.6) μ.
- On nutrient agar plus 2 per cent dextrose; culture 7 days old:
  - Macroconidia—
    - 3-septate.—38.1 by 4.08 (30.6 to 45 by 3.6 to 4.5) μ.
- On nutrient agar plus 2 per cent dextrose; culture 17 days old:
  - Macroconidia—
    - 3-septate.—38.4 by 4.16 (34.2 to 41.4 by 3.6 to 4.9) μ.
- On 2 per cent potato agar without sugar; culture 60 days old:
  - Macroconidia—
    - 1-septate.—34 by 3.6 μ. Rare.
    - 3-septate.—40.1 by 4.52 (34.2 to 49 by 4 to 4.9) μ. Up to 75 to 80 per cent.
    - 4-septate.—43.2 by 4.81 (37.8 to 48.6 by 4 to 4.9) μ. Up to 15 to 20 per cent.
    - 5-septate.—46.8 by 4.9 (41.4 to 54 by 4.9 to 4.9) μ. Less than 1 per cent.
  - Chlamydospores—
    - In spores.—Terminal, 6.3 by 5.85 (5.4 to 7.2 by 5.4 to 6.3) μ.
- On 2 per cent potato agar without sugar; culture 7 days old:
  - Macroconidia—
    - 3-septate.—46.7 by 4.2 (41.4 to 52.2 by 3.6 to 4.5) μ.
- On 2 per cent potato agar without sugar; culture 22 days old:
  - Macroconidia—
    - 3-septate.—37.6 by 4.53 (27 to 46.8 by 4 to 5) μ.
    - 5-septate.—45 by 4.9 μ. Rare.

- On potato agar without sugar; culture 14 days old:
  - Macroconidia—
    - 3-septate.—40.86 by 4.74 (37.8 to 45 by 4.5 to 4.9) μ. Up to 90 to 95 per cent.
    - 4-septate.—45.3 by 4.8 (43.2 to 46.8 by 4.5 to 5) μ. Up to 50 per cent.
    - 5-septate.—48.6 by 5.1 μ. Rare.
  - Microconidia—
    - 0-septate.—8.91 by 3.17 (7.2 to 9.9 by 2.7 to 3.1) μ. Up to 95 per cent.
    - 1-septate.—9.9 by 4.05 (9 to 10.8 by 3.6 to 4.5) μ. Up to 5 per cent.
- On *Melilotus alba* stems; culture 13 days old:
  - Macroconidia—
    - 3-septate.—38.9 by 4.7 (30.6 to 45 by 4 to 5.4) μ. Up to 80 to 90 per cent.
    - 4-septate.—40.2 by 5.2 (37.8 to 43.2 by 4.9 to 5.4) μ. Up to 5 per cent.
    - 5-septate.—48.6 by 5 μ. Only 1 spore measured; rare.
  - Microconidia—
    - 0-septate.—10 by 3.64 (7.2 to 12.6 by 3.1 to 4.5) μ.
    - 1-septate.—15 by 3.8 (13.5 to 16.2 by 3.6 to 4.5) μ.
- On oat agar; culture 7 days old:
  - Macroconidia—
    - 3-septate.—38.9 by 4.7 (30.6 to 45 by 4 to 4.9) μ. Up to 80 to 90 per cent.
    - 4-septate.—48 by 4.8 (41.4 to 52.2 by 4.5 to 4.9) μ. Up to 10 to 20 per cent.
    - 5-septate.—52.2 by 4.9 μ. Rare.
- On corn-meal agar; culture 27 days old:
  - Macroconidia—
    - 3-septate.—37.8 by 4.59 (23.4 to 45 by 4 to 4.9) μ.
    - 4-septate.—42.1 by 4.8 (39.6 to 46.8 by 4.5 to 4.9) μ.
    - 5-septate.—49.5 by 4.9 (46.8 to 52.2 by 4.9) μ.
    - 6-septate.—54 by 5.4 μ. 1 spore only.
  - Chlamydospores, in mycelium—
    - 0-septate.—9.36 by 9.72 (7.2 to 10.8 by 7.2 to 10.8) μ.
- On Lima-bean agar; culture 7 days old:
  - Macroconidia—
    - 3-septate.—41.9 by 4.65 (30.6 to 48.6 by 4 to 4.9) μ. Up to 80 to 90 per cent.
    - 4-septate.—48.6 by 4.9 μ. Up to 10 to 20 per cent.
- On steamed rice; culture 21 days old:
  - Macroconidia—
    - 3-septate.—42.9 by 4.4 (37.8 to 46.8 by 4 to 4.9) μ. Up to 95 per cent.
    - 4-septate.—45.5 by 4.34 (39.6 to 48.6 by 4 to 4.9) μ. Up to 5 per cent.
    - 5-septate.—46.8 by 4.7 μ. Rare.
  - Microconidia—
    - 0-septate.—8.8 by 3.18 (7.2 to 10.8 by 2.7 to 3.6) μ. Up to 95 per cent.
    - 1-septate.—9.9 by 3.6 μ. Up to 5 per cent.
- Average of the above-listed measurements:
  - Macroconidia—
    - 1-septate.—34 by 3.6 μ.
    - 2-septate.—37 by 4.0 μ.
    - 3-septate.—40 by 4.4 μ.
    - 4-septate.—44.5 by 4.84 μ.
    - 5-septate.—46.8 by 4.92 μ.
    - 6-septate.—51.3 by 5.4 μ.
  - Microconidia—
    - 0-septate.—8.95 by 3.43 μ.
    - 1-septate.—11.9 by 3.89 μ.
  - Chlamydospores—
    - In spores.—(a) Intercalary, 8.5 by 7.46 μ; and (b) terminal, 6.25 by 5.4 μ.
    - In mycelium.—(a) Intercalary, 9.72 by 9.36 μ; and (b) terminal, 11.3 by 11.1 μ.

The cultural and morphological description given above shows clearly that the alfalfa-wilt fungus belongs to the section Elegans of the genus Fusarium. A comparison with illustrations and descriptions of other species of this section by Wollenweber (*23*, *24*), Sherbakoff (*17*), and others convinced the writer that it resembled *F. oxysporum* Schlecht. emend. Wr. (*13, p. 139; 23*) so closely that it can best be referred to a variety of this species.[4]

Fusarium oxysporum var. *medicaginis* differs from *F. oxysporum* in pathogenicity and in having both longer and slightly wider spores. Its spores differ little in width from those of *F. oxysporum* var. *gladioli* and *F. oxysporum* var. *nicotianae* as described by Massey (*9*) and Johnson (*4*), respectively, but average somewhat longer.

PATHOGENICITY

The parasitism of *Fusarium oxysporum* var. *medicaginis* was proved by inoculating seed and plants and by infesting the soil with it.

On September 30, 1926, inoculations were made as follows:

(*a*) Sixteen 1-year-old alfalfa plants of the Kansas common variety that had always been grown in sterilized soil in the greenhouse were transplanted to sterilized soil and inoculated by pouring a spore suspension about their crowns. No wounds were made purposely, but rootlets were broken when the plants were transplanted.

(*b*) Sixteen plants of the same lot were transplanted and inoculated by inserting spores and hyphae beneath the bark through wounds.

(*c*) Six plants of the same lot were transplanted in a similar manner. Three of these were wounded like those described above but were not inoculated, and the other three were held unwounded except for the broken rootlets. The unwounded ones constituted the controls.

(*d*) Three pots of sterilized soil were sown with Kansas common seed. Two of these were inoculated by pouring a spore suspension over the seed before it was covered, and the third was held uninoculated as a control.

The first plant to show evidence of infection was pulled on November 1. Infection had been apparent for some time, as the disease was well advanced at this time. This plant is shown in Figure 1. Typical vascular browning was present both in the root and for some distance up the stem. The Fusarium was recovered from the internal brown tissue, studied in culture, and later used for further inoculation tests with positive results. On November 15 infection was evident in 12 of the 15 plants (1 plant did not survive transplanting) inoculated by inserting the inoculum into wounds. On December 10, when the plants were taken up and examined, various amounts of vascular browning were found in both the roots and stems in all but 1 plant, and the causal fungus was recovered from them all. Hence a total of 14 out of 15 plants, or 93.3 per cent, became infected. There was no evidence of infection in any of the controls. On November 15, 10 of the 14 plants (2 plants did not survive transplanting) inoculated with a spore suspension (*a*) showed unmistakable symptoms of the disease. Two more, making a total of 12 out of 14, or 85.7 per cent, became infected later and the fungus was recovered from all of them. Although infection became evident a little sooner when the inoculum was inserted into wounds, the ultimate amount of infection was nearly the same.

Infection in the seedlings (*d*) was first definitely proved to have taken place on November 26, although some plants had died previously, supposedly from damping off but probably from wilt. Isolations were made from two wilted plants on this date and the causal organism recovered. The leaves of the affected plants turned yellow and then brown, and death resulted. This phase of the experiment was discontinued January 5, 1927, at which time 17 and 40 per cent, respectively, of the seedlings in the two inoculated pots were infected, and the causal fungus was recovered from them. At this time four plants in the uninoculated control pot also showed symptoms of a similar nature, and the fungus was recovered from three of them. These pots had stood near one another for more than two months, and no doubt some inoculum had been splashed from the inoculated pots into the control one by watering.

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[4] The writer is indebted to Dr. C. D. Sherbakoff, who after examining cultures of the fungus agreed with him that the fungus might well be made a variety of *Fusarium oxysporum* Schlecht. emend. Wr.

This experiment shows that infection can be produced in 1-year-old plants as well as in seedlings either by inserting the fungus into wounds or by infesting the soil. Infection appeared first in about a month, and affected plants were frequently entirely dead in six weeks. Symptoms similar to those evident in the field are produced under greenhouse conditions.

On February 1, 1927, plants of Kansas common alfalfa grown in the field from seed sown in August were brought to the greenhouse and transplanted into sterilized soil. Part of these plants were inoculated by inserting hyphae and spores into wounds in the taproot just below the crown and part by pouring a spore suspension into the soil about them. The fungus used for the inoculations was a single-spore culture of the fungus used in the experiment detailed above. Only 5 to 10 per cent of the plants in this test became infected, a slightly higher percentage occurring in those plants inoculated in wounds. This test was run largely during the winter and early spring months, and it has been observed since that only a small percentage of infection takes place during the winter months, whereas 75 to 100 per cent will occur during the late spring, summer, or autumn months. For example, on October 12, 1927, an experiment was begun in which this same strain of the fungus was inoculated into 10 plants, all of which became infected before December 12, when the experiment was discontinued. None of the controls became infected.

A culture of the Fusarium obtained from one of the plants infected in the first experiment cited above was used for a parasitism test made May 18, 1927. The plants used in this case were 9 months old and had grown in the field until two months prior to their inoculation, when they had been set in pots in the greenhouse. The tops were cut back, the soil was removed from the crowns and the upper parts of the taproots, and the inoculum was inserted beneath the bark of the taproot through wounds. Two pots each containing five plants were used for the inoculations, and a like number used as controls were injured, but not inoculated. All but two of the inoculated plants, or 80 per cent, became infected, while the controls remained healthy. The fungus was recovered from the diseased tissue and its identity determined.

Many more inoculation tests have been made with these and other isolations of this fungus over a period of one and one-half years, and usually a large percentage of infection resulted. However, it is believed that the tests detailed are sufficient to prove that this fungus is parasitic to alfalfa.

Since the alfalfa is so closely related to *Fusarium oxysporum*, infection experiments were conducted in which 10 alfalfa plants were inoculated with *F. oxysporum* and 10 young potato plants were inoculated with the Fusarium from alfalfa. In no case did any infection result. This test, although not carried out on a very large scale, indicates that the Fusarium from alfalfa differs parasitically from the one from potato. Since the fungus (*F. hyperoxysporum*) that causes stem rot of the sweet potato is so closely related to *F. oxysporum*, the parasitism of the Fusarium from alfalfa was also tested on sweet potato, but with negative results.

Alfalfa is used regularly in rotation with potatoes in Kansas, and although there is a varying amount of Fusarium wilt in the potatoes each year, none has ever been found in alfalfa in that State. Furthermore, as yet there is no evidence that the wilt of alfalfa occurs in any of the Northern States, in many of which both alfalfa and potatoes are used in rotation. Since the Fusarium from alfalfa seems to have such a limited distribution and *F. oxysporum* is so widely distributed it seems certain that they are at least parasitically two different organisms.

LIFE HISTORY

Little is known regarding the life history of *Fusarium oxysporum* var. *medicaginis*. So far as the writer has observed, the disease produced by it is present in about the same amount throughout the growing season. No doubt the causal organism is spread from one plant to another largely by the soil water. Having come in contact with a healthy plant, it apparently can enter through wounds or directly through the epidermis, especially of small rootlets. No experiments have been conducted to prove definitely that the fungus can enter the roots except through wounds, but this is indicated by the fact that the seedlings growing in soil that was sterilized and then infested become infected readily. The fungus often enters the taproot by way of small lateral roots. Frequently small rootlets having a brown color have been followed to the larger roots or the taproot and the infection thus traced to the larger roots, from which the fungus was isolated. After reaching the main roots from the rootlets the fungus may grow up or down within the vascular system. In several cases observed the vascular discoloratión was more pronounced toward the top, indicating perhaps that at least in some instances the fungus makes more rapid progress upward.

Once the fungus has gained entrance to the main taproot, symptoms of the disease may appear in the tops in about a month, although it may take much longer. The fungus passes from the root into the stems, in which a conspicuous browning of the vascular region, often extending upward for several inches, is produced. After gradually spreading throughout the taproot and into the rootlets, the fungus finally causes the entire woody cylinders of the roots of small plants to turn dark in color and eventually kills them. The roots decay and the fungus is liberated. Plants grown on this infested soil become infected and thus the disease continues to spread.

CONTROL

No experiments have been conducted with a view to determining control measures. Observations made on some of Westover's fertilizer plots at West Point, Miss., did not indicate that lime or superphosphate (acid phosphate) had any effect on the prevalence of the disease.

Likewise the disease could be found in practically every variety of alfalfa grown in Westover's trials, although in many instances very few diseased plants were seen. Kansas common, Grimm, and Hairy Peruvian varieties have been inoculated in greenhouse tests and appear to be about equally susceptible.

How long the fungus can live in the soil is not known. Judging from the knowledge of other Fusaria of this type, no doubt a rather long rotation will be necessary to eliminate this source of infection.

SUMMARY

A disease of alfalfa in every way similar to the Fusarium-wilt diseases of many other plants is herein described. The disease is known to occur only in Mississippi, although plants received from California appeared to have a very similar and probably the same disease. Because of its limited distribution the disease is not of very great economic importance at the present time. However, it is a factor in thinning out the stands of alfalfa in northeastern Mississippi, where as many as 15 per cent of infected plants have been found in a field, and no soil or climatic factors that would prevent the spread of this disease to other regions are known.

The leaves of affected plants turn yellow, the tips of the stems frequently wilt, and the plants eventually die. Frequently one or more stems die while the rest remain healthy for a longer time. The vascular region of diseased plants, especially of the taproot, is some shade of brown, usually ranging from cinnamon brown to mummy brown.

This Fusarium wilt resembles bacterial wilt due to *Aplanobacter insidiosum* L. McC. to some extent but is readily distinguished from it.

The causal organism is described as *Fusarium oxysporum* var. *medicaginis*, n. var. It differs from *F. oxysporum* Schlecht. emend. Wr. chiefly in parasitism and spore size. It also seems to have a much more limited distribution than *F. oxysporum*. Its morphological and cultural characters are described in detail.

No control measures have been worked out.

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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON PIGMENT PATTERNS IN VARIETIES OF COMMON BEANS[1]

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INTRODUCTION

The failure to appreciate the rôle played by environment in the development of seed-coat pigments was most forcibly illustrated when the soy bean suddenly became an important crop in the Corn Belt. Some time passed before agronomists came to appreciate the fact that in a study of pigmentation environmental conditions must be considered as well as heredity; but no doubt it is now generally understood that not all mottling is the result of hereditary factors.

During the course of rather extensive experiments with mottling in soy beans (*2*),[2] the senior writer became interested in the general applicability of the same studies to various other leguminous plants and in particular to certain varieties of the common bean (*Phaseolus vulgaris*).

Thirteen years ago Pearl and Surface (*3*) made a careful study of the market situation with regard to varieties of common beans sold for baking purposes, particularly the Old Fashioned Yellow Eye and the Improved Yellow Eye varieties. The preference for a bean of a particular pattern as described at that time is very interesting in the light of the information now available. Had the variability due to environmental influences been better understood it is possible that the market demands might in many cases have been less exacting.

The production of seed-coat pigments forms a complicated study and the chemical and physiological processes involved are none too well understood at the present time, but the studies here reported throw some light on the rôle played by environmental factors and will perhaps be of some practical significance.

VARIETIES USED

During the course of two years' work (1926 and 1927) the following varieties of beans were studied: Old Fashioned Yellow Eye, Small Yellow Eye, Golden Wax, and Golden Carmine.

The color patterns of these varieties are shown in Figure 1, where the different classes represent artificial standards for convenience in statistical comparisons. The first three varieties are eyed types with a background of opaque white. The color patterns of the Old Fashioned Yellow Eye and the Small Yellow Eye are yellow and that of the Golden Wax is a mottled purple. The Golden Carmine is an entirely

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[1] Received for publication July 14, 1928; issued November, 1928. This paper is No. 185 of the Biological Laboratory, Maine Agricultural Experiment Station, and No. 92 of the Department of Genetics, Wisconsin Agricultural Experiment Station. The work here recorded was begun at the Wisconsin station by the senior author under the supervision of R. A. Brink, but the present report is largely the result of the efforts of Mrs. Burgess, who has taken charge of the cooperative work at Monmouth, Me., and of C. R. Burnham, who has carried on the tests at Madison, Wis.

[2] Reference is made by number (italic) to "Literature cited," p. 442.

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different type with peculiar red streaks on a somewhat yellowish pink background.

The Old Fashioned Yellow Eye has had a most interesting history in the bean-growing districts of Maine (*3*). The typical color pattern (see Maine-grown beans, fig. 2) of this variety is within itself a source of attraction, and it is natural that a prejudice should have become established against beans that depart too widely from this type. Some difficulty has been experienced in always securing this uniform pattern, but nevertheless it is quite definite in outline and is much more uniform than that of many other varieties.

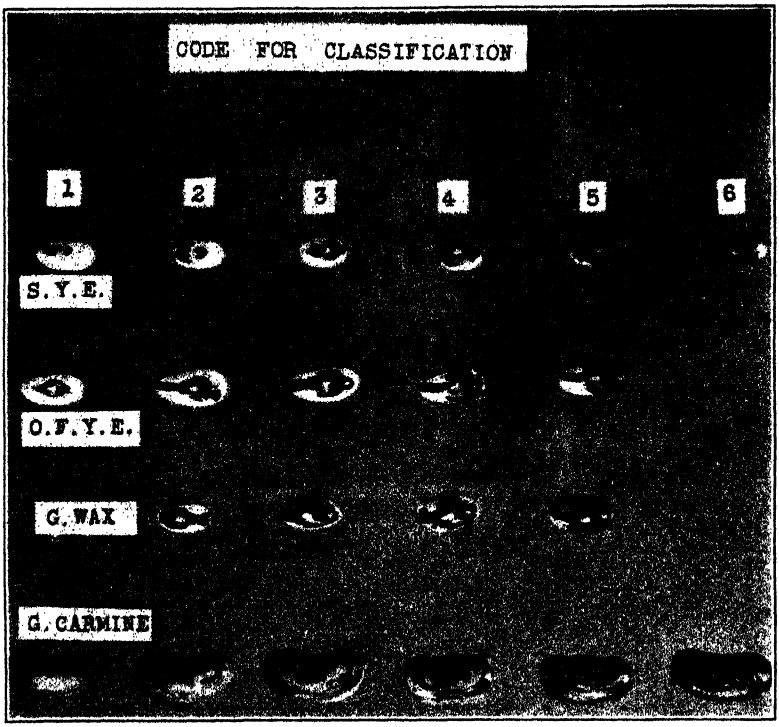

FIG. 1.—Arbitrary pattern classes of bean pigmentation adopted by the writers to facilitate the statistical analysis of their results. Class 2 shows the typical patterns of the Old Fashioned Yellow Eye variety; classes 3 and 4 are more typical of the Golden Wax. For the first three varieties classes 5 and 6 show what extreme modifications may be brought about by simply changing the environmental conditions. For the Golden Carmine variety the situation is somewhat different. Classes 1 to 4 are typical of the variety, and classes 5 and 6 occur very sporadically and apparently quite independent of environmental conditions. About three-fourths natural size. Photographed by H. C. White

The Old Fashioned Yellow Eye variety has been studied extensively at the Maine station (*6*, *7*), and Karl Sax has considerable unpublished data on the variability of size of bean and of the color pattern. Although many of these studies have been somewhat incidental to the present subject, they have helped to establish familiarity with the influence of hereditary factors on the amount of pigment formed. Progeny records within pure lines constitute the best information at hand, and records kept by Sax and Burgess give a pretty definite idea of the possibility of selecting for more uniformity in this variety. Slight differences no doubt exist between different lines and it has been suspected that hereditary influences control the variability on a single plant to some extent, but the progress of these studies has been handicapped because of the large amount of variability due to environment.

The Small Yellow Eye is not a commercial variety, but is the result of a selection made by one of the writers (Burgess) in 1923. It is an offspring from a hybrid between the Improved Yellow Eye and a white variety (*4*, *5*), and it is one of the very few segregates that approached the Old Fashioned Yellow Eye in pattern type with any degree of exactness. Experience has proved, however, that it is somewhat more variable than the Old Fashioned Yellow Eye.

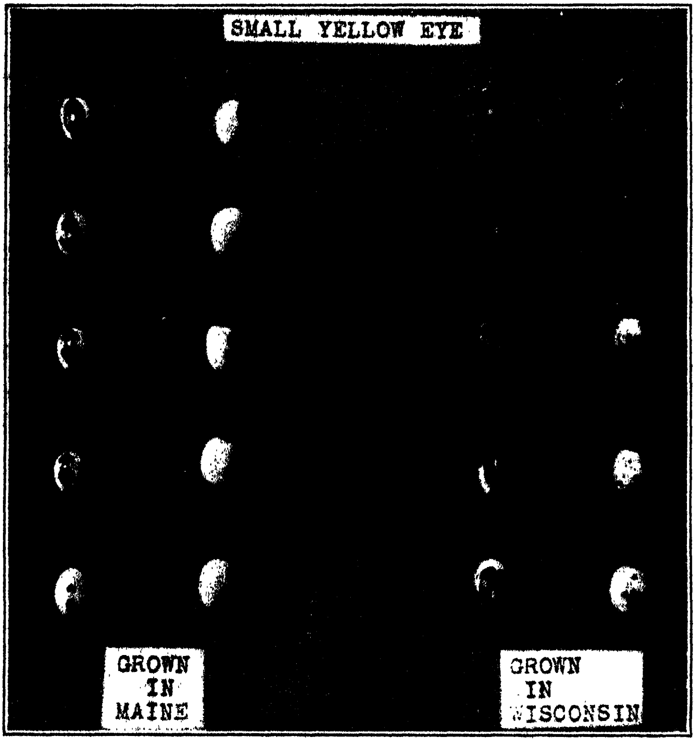

FIG. 2.—Extreme color pattern differences in Small Yellow Eye beans grown under different environmental conditions: A, Beans from plants grown on sandy soil at Monmouth, Me.; B, beans from plants grown on fertile soil at Madison, Wis., spaced 3 feet apart. As here shown, the color pattern of the Small Yellow Eye variety was very readily influenced by environment. Plants spaced 3 feet apart on rich soil displayed the extreme amount of pigment. Difference in climate seemed to be of minor importance, since dark beans were obtained at Wisconsin only when the plants were spaced 3 feet apart and grown on rich soil. Three-fourths natural size. Photographed by H. C. White

The Golden Wax and Golden Carmine seeds were selected at random from commercial varieties that were being grown at the Wisconsin station. Occasional dark colored beans are produced by the Golden Carmine variety, but these occur only sporadically, as if due to some peculiar change in the cells of somatic tissue rather than to either environment or normal modes of inheritance.

TESTING THE EFFECT OF ENVIRONMENT

A study of the results obtained with the four varieties of beans grown in different regions and under different soil conditions gives a vivid picture of the effect produced by environment; and for the Small Yellow Eye variety, at least, the results are striking.

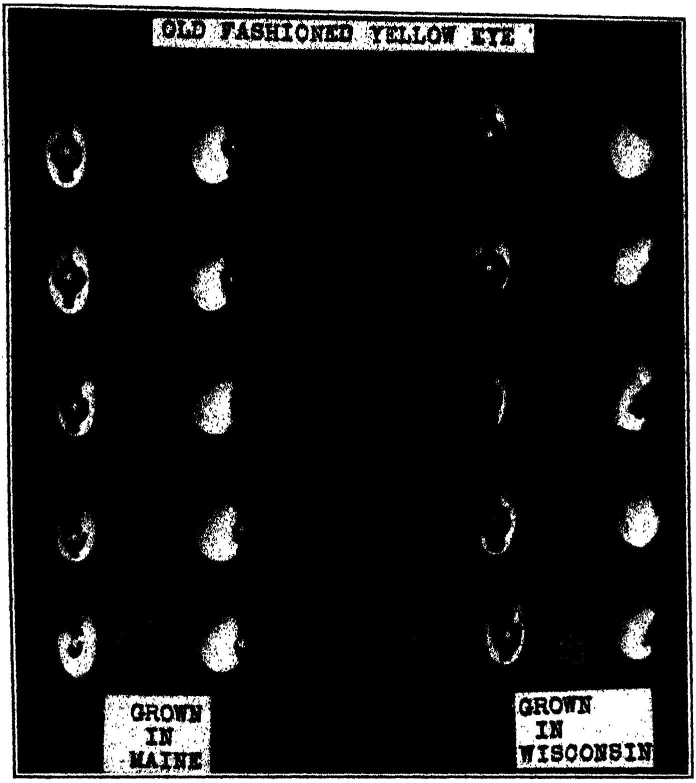

FIG. 3.—A very stable color pattern changed by environmental conditions: A, Old Fashioned Yellow Eye beans from plants grown on sandy soil at Monmouth, Me.; B, beans of the same variety from plants grown on fertile soil at Madison, Wis., spaced 3 feet apart. The Old Fashioned Yellow Eye beans were markedly affected by the rich soil of Wisconsin, but the difference was not nearly so striking as in the case of the Small Yellow Eye variety. This variety might well be considered remarkable for its uniformity of pattern. Three-fourths natural size. Photographed by H. C. White

The environmental conditions under which the beans were grown in 1926 were as follows:

Madison, Wis.	sandy soil	plants 1 inch apart in 3-foot rows. plants 3 feet apart in 3-foot rows.
	very rich loam soil	plants 2 inches apart in 3-foot rows. plants 3 feet apart in 3-foot rows.
Highmoor Farm, Monmouth, Me.	sandy soil	plants 1 inch apart in 3-foot rows. plants 2 feet apart in 3-foot rows.
	very rich loam soil	plants 2 inches apart in 3-foot rows. plants 2 feet apart in 3-foot rows.
Aroostook Farm, Presque Isle, Me.		plants 2 feet apart (for the Old Fashioned Yellow Eye and Small Yellow Eye varieties).

The varieties tested were not necessarily pure lines, but before any samples were taken a composite lot of seed was mixed thoroughly and subsequent samples were made from this common lot so that heriditary factors should be comparable for each environment.

Environment seems to have had little effect on the Golden Wax and Golden Carmine varieties, but undoubtedly it exerted considerable effect on the Old Fashioned Yellow Eye and even more on the Small Yellow Eye. Figures 2 and 3 give very good illustrations (not exaggerated) of the difference between the beans grown on sandy soil at Monmouth, Me., and those grown on very rich soil at Madison, Wis. The tremendous increase in pigment due to the rich soil of Wisconsin is clearly apparent.

NUMERICAL INTERPRETATION OF RESULTS

To put the results in numerical form, six arbitrary classes were made according to the amount of pigment present on each bean. (See fig. 1.) It must be kept in mind that these artificial classes intergrade to some extent, so that a statistical treatment may be somewhat misleading except where the differences are very large. The χ^2 test is not held to be a true measure of significance because the personal error in classification alone is an extremely variable factor; but it does help to visualize the data. The χ^2 values for all of the tabulations presented here ($\chi^2=40$ is the lowest) represent infinite odds according to Elderton's (*1*) tables, but since the personal error also affects the value of χ^2 no attempt should be made to distinguish fine differences. Furthermore the extreme contrasts are the most important, and these hardly need statistical interpretation.

The most obvious contrast is between the Small Yellow Eye beans grown on rich soil at Madison, Wis., and those grown on unfertilized soil at Aroostook Farm, Presque Isle, Me., as shown in Table 1.

TABLE 1.—*Effect of fertility of soil on pigmentation of Small Yellow Eye beans*

Environment	Per cent of total number of seeds observed in class—						Total number of seeds
	1	2	3	4	5	6	
Rich soil, Madison, Wis., plants 3 feet apart	1.2	6.4	16.7	27.6	37.2	10.9	952
Very infertile soil, Presque Isle, Me., plants 2 feet apart	41.6	5.9	35.3	17.1			269

No statistical analysis is needed to portray the significance of the figures shown in Table 1, but if the χ^2 test is applied on the assumption that the Maine-grown beans are the result of chance variation from the Wisconsin beans, $\chi^2=3{,}895$, and the probability is so infinitesimally small that the certainty of the difference can not be questioned.[3]

The distance of spacing was also an important factor, especially on the rich soil at Madison. The figures observed for the Small Yellow Eye and the Old Fashioned Yellow Eye varieties are shown in Tables 2 and 3.

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[3] χ^2 has been calculated from the actual frequencies. The total number of frequencies in this instance is 269 and the comparison is between the actual frequencies for the beans grown in Maine and a theoretical population (also of 269 frequencies) distributed in accord with the classification of the beans grown in Wisconsin.

TABLE 2.—*Effect of thickness of stand on pigmentation of Small Yellow Eye beans*

Environment	Per cent of total number of seeds in class—						Total number of seeds
	1	2	3	4	5	6	
Plants 3 feet apart on rich soil at Madison, Wis.	1.2	6.4	16.7	27.6	37.2	10.9	962
Plants 2 inches apart on rich soil at Madison, Wis.	39.4	24.6	25.4	10.6			264

$\chi^2=3{,}478$

TABLE 3.—*Effect of fertility of soil and thickness of stand on pigmentation of Old Fashioned Yellow Eye beans*

Environment	Per cent of total number of seeds in class—						Total number of seeds	
	1	2	3	4	5	6		
Rich soil, Madison, Wis., plants 3 feet apart		46.0	35.5	12.0	6.5		722	χ^2=infinite.
Sandy soil, Monmouth, Me., plants 2 feet apart	23.6	47.3	29.2				641	
Plants 3 feet apart on rich soil at Madison, Wis.		46.0	35.5	12.0	6.5		722	χ^2=40.
Plants 2 inches apart on rich soil at Madison, Wis.		55.4	38.8	4.4	1.4		363	

Differences in the Old Fashioned Yellow Eye are very significant when the beans grown in Wisconsin on rich soil are compared with those grown in Maine. The effect of distance of spacing, however, was less striking than in the case of the Small Yellow Eye.

The effect of these factors on the pigmentation of the Golden Wax variety is shown in Table 4. This variety seems to be naturally somewhat variable, and although the differences brought about by environment are probably significant, the contrast is not nearly so great as in the Yellow Eye variety.

TABLE 4.—*Effect of fertility of soil and thickness of stand on pigmentation of Golden Wax beans*

Environment	Per cent of total number of seeds in class—						Total number of seeds	
	1	2	3	4	5	6		
Rich soil, Madison Wis., plants 3 feet apart		1.8	17.6	31.6	48.9		272	χ^2=216.
Sandy soil, Monmouth, Me., plants 2 feet apart		1.8	46.5	34.9	16.8		720	
Plants 3 feet apart on rich soil at Madison, Wis.		1.8	17.6	31.6	48.9		272	χ^2=223.
Plants 2 inches apart on rich soil at Madison, Wis.		5.0	28.6	47.8	18.6		161	

A tabulation for the Golden Carmine variety showed no definite effect due to environment. The occurrence of the very dark seeds (fig. 1, classes 5 and 6) was relatively infrequent and seemed to be quite independent of environmental influences.

Although the 1926 results were very convincing and proved practically conclusive that the rich soil at Madison was responsible for the greater quantity of pigment present in the Small Yellow Eye and the Old Fashioned Yellow Eye varieties, it was thought well to confirm these results by an additional test, and this was carried out during the season of 1927. The dark colored Small Yellow Eye beans produced on the rich soil at Madison in 1926 and beans of the typical varietal patterns grown on the sandy soil at Monmouth, Me., in 1926 were planted on the same soil at Monmouth, Me. The object of this test was to determine whether or not the dark beans would show a tendency to perpetuate their kind. The results are given in Table 5.

From this table it is seen that the highly colored Wisconsin seed failed to reproduce even as much pigment as the Maine-grown seed. It is reasonable to suppose, therefore, that the extreme effects which were visible in 1926 were due entirely to environmental influences.

TABLE 5.—*Inheritance of pigmentation in beans grown in 1927 at Monmouth, Me., from highly colored and from normal seed produced under different environmental conditions*

Source and pigmentation of seed	Per cent of total number of seeds in class—						Total number of seeds
	1	2	3	4	5	6	
Highly colored seed from rich soil at Madison, Wis., 1926	11.1	31.8	47.5	8.8	0.8	0.1	1,808
Normal seed from sandy soil at Monmouth, Me., 1926	7.8	18.4	54.4	19.1	0.3		1,580

$\chi^2 = 318.$

INTERPRETATION OF ENVIRONMENTAL INFLUENCES

What the controlling influences are that affect pigment formation in a seed coat is a difficult question and the reader is referred to the previous studies which attempt to interpret certain observations for soy beans (*2*). Undoubtedly the accumulation of sugars is a very important factor because of the glucosidal nature of the pigments. But the combination of environmental factors responsible for an accumulation of sugars may be very complex. A particular feature of the plant's environment naturally produces effects according to the combination of other environmental factors and the physiological condition within the plant must be considered at all times.

The most important environmental factors that affect the production of seed-coat pigments are probably related to the nourishment of the plant. A rich soil has usually increased the production of pigment in seed coats. Spacing the plants a considerable distance apart also seems to have the same effect. It is difficult always to duplicate experiments, however, and it seems that the nature of maturity is one of the most important things to be considered. Prolonged vegetative growth may upset all previous expectations, and it is believed that plants of the runner type (*7*) which ripen unevenly are more likely to show variability within individuals.

For the maximum development of pigment a combination of factors is necessary, and unfortunately these factors are rather poorly understood. On certain types of soil it makes little difference how the plants are spaced. In fact, in certain instances, results contradictory to the general rule have been observed. For instance, during the summer of 1927 somewhat more pigment was formed in Old Fashioned Yellow Eye and Small Yellow Eye beans at Highmoor Farm spaced 2 inches apart than in those spaced 3 feet apart. Probably there was some difference in soil fertility, but whatever may have been the cause it is outside the realm of the present discussion. The main point to consider here is that environmental factors are important in determining the amount of seed-coat pigment that is produced.

The results here recorded are very similar to those that have many times been found in the course of the soy-bean studies. It is evident that a large number of environmental influences indirectly affect the internal condition of the plant, such as soil fertility, weather conditions, soil moisture, and distance between plants. With this in mind it is easy to understand how one limiting factor may be responsible for the difference between lightly colored and highly colored beans in one instance while the same factor may be insignificant in another instance. For further progress along this line of investigation a more intimate knowledge of the physiological conditions within the plant is essential.

SUMMARY

Environmental conditions are described which have been found to be of great importance in determining color patterns in common beans. Four varieties of beans were tested in different types of soil in Maine and Wisconsin.

Plants spaced 3 feet apart on rich soil at Madison, Wis., developed a striking amount of pigment. That this was not the result of climatic differences is apparent from the fact that dark beans were obtained at Wisconsin only when the plants were spaced 3 feet apart and grown on rich soil.

To determine whether the dark beans would tend to perpetuate their kind, dark colored Small Yellow Eye beans produced in 1926 on the rich soil at Madison and beans of the typical varietal patterns grown on sandy soil at Monmouth, Me., were planted on the same soil at Monmouth in 1927. In these experiments the highly colored Wisconsin seed failed to produce even as much pigment as the Maine-grown seed. It is therefore believed that the extraordinary amount of pigment present in the Wisconsin seed in 1926 was due entirely to environmental influences.

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A PYTHIUM SEEDLING BLIGHT AND ROOT ROT OF DENT CORN[1]

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INTRODUCTION

During the course of investigations of the corn rots caused by *Diplodia zeae*, *Gibberella saubinetii*, and *Fusarium moniliforme* certain phases of the root-rot problem made it increasingly apparent that in all probability other organisms than these named are to be considered important causes of the diseases.

The cortical rots produced by the three fungi previously mentioned are confined chiefly to the coleorhiza and the mesocotyl and spread from them into the cortex of the primary and adventitious roots of the corn seedling. The development of the cortical rotting is in marked contrast to a distinct type of root rotting that begins at the tips of the rootlets and reaches the mesocotyl only in the late stages of seedling blight.

In 1923, when a species of Pythium was isolated from the roots of corn plants grown in the field at Madison, Wis., and from seedlings that had blighted in unsterilized soil in the greenhouse, the present investigation was started to determine to what extent, if any, Pythium is a factor in the corn root-rot and seedling-blight problems. The study also included a comparison of the symptoms produced under controlled conditions with those observed in rotting rootlets in the field. Since 1923 experiments have been conducted in the greenhouse and in the field at Madison, Wis., and in the field at Bloomington, Ill.

PYTHIUM INJURY

The severity of the Pythium injury depends upon environmental conditions. Germination may be prevented at low temperatures (12° to 16° C.), particularly in wet soils, by the rapid rotting of the embryo during the period of imbibition of moisture. A water-soaked appearance gives evidence of the presence of the fungus within the

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[2] The writers wish to express their appreciation to Dr. C. R. Ball and Dr. A. G. Johnson for helpful criticisms in the preparation of the manuscript and to acknowledge with thanks the laboratory facilities furnished by the Illinois Weslyan University during June, 1926, and especially the courtesies extended during that time by Dr. Ella Martin.

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embryo. Discoloration of the water-soaked tissue follows the entrance of secondary organisms. Under conditions less favorable for the development of the disease, a soft rot of the seedling root system may develop, resulting in the gradual reduction of the functioning roots. In such plants subsequent invasion of the mesocotyl occurs and seedling blight results. Or, a mild root rot may occur, not severe enough to kill the seedling, yet resulting in a reduction in size and vigor, due to the decreased root surface and perhaps to the effect of toxins produced in the invaded cortical areas.

The influence of such stunting upon the final yield has been demonstrated by Holbert and his coworkers (*7*).[3] They state that, under field conditions, a direct correlation exists between early height and vigor and yield of corn plants.

The greenhouse and field experiments have shown that Pythium injury is unlike that caused by *Diplodia zeae* and *Gibberella saubinetii* in that Pythium does not attack the mesocotyl of the corn seedling except occasionally in severe cases of invasion. Although the roots may be severely rotted, the mesocotyl usually remains clean. The experiments have shown that infection occurs near the tips of the rootlets and travels upward, much as described by Harter for the Pythium rootlet rot of sweet potatoes (*6*). The fungus produces a soft rot first in the root cortex and later in the vascular elements. There is no marked discoloration of these invaded tissues before secondary organisms make their entrance into the water-soaked tissue. Lobulate sporangia occur in the cortex, usually near the surface, and frequently appear in the root hairs. (Pl. 1, F, G.) It is exceedingly difficult to trace the course of the fungus through the root, because the granular protoplasm moves from the older mycelium into the tip as hyphal invasion progresses. The position of the fungus in the cortex is evident chiefly by the presence of the lobulate sporangia, as the thin-walled hyphae are practically invisible. Later conidia and oospores may be found within the vascular tissue as well as in the cortex of the root. The fruiting bodies ordinarily appear in greater numbers in rootlets grown at low soil temperatures.

In order to secure satisfactory material for study, it was necessary to use great care in the removal of infected plants from the soil in the greenhouse and in the field, so as to prevent breaking and loss of the rotted rootlets. Their recovery from large plants in the field was particularly difficult and uncertain.

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[3] Reference is made by number (italic) to "Literature cited," p. 464.

EXPLANATORY LEGEND FOR PLATE 1

A.—Zoosporangia from corn rootlets: *a*, before and, *b*, after zoospore formation. × 200.

B.—Oospore within the cortex of a corn seedling root, grown on a plate of soil-decoction agar in an ordinary refrigerator. × 400.

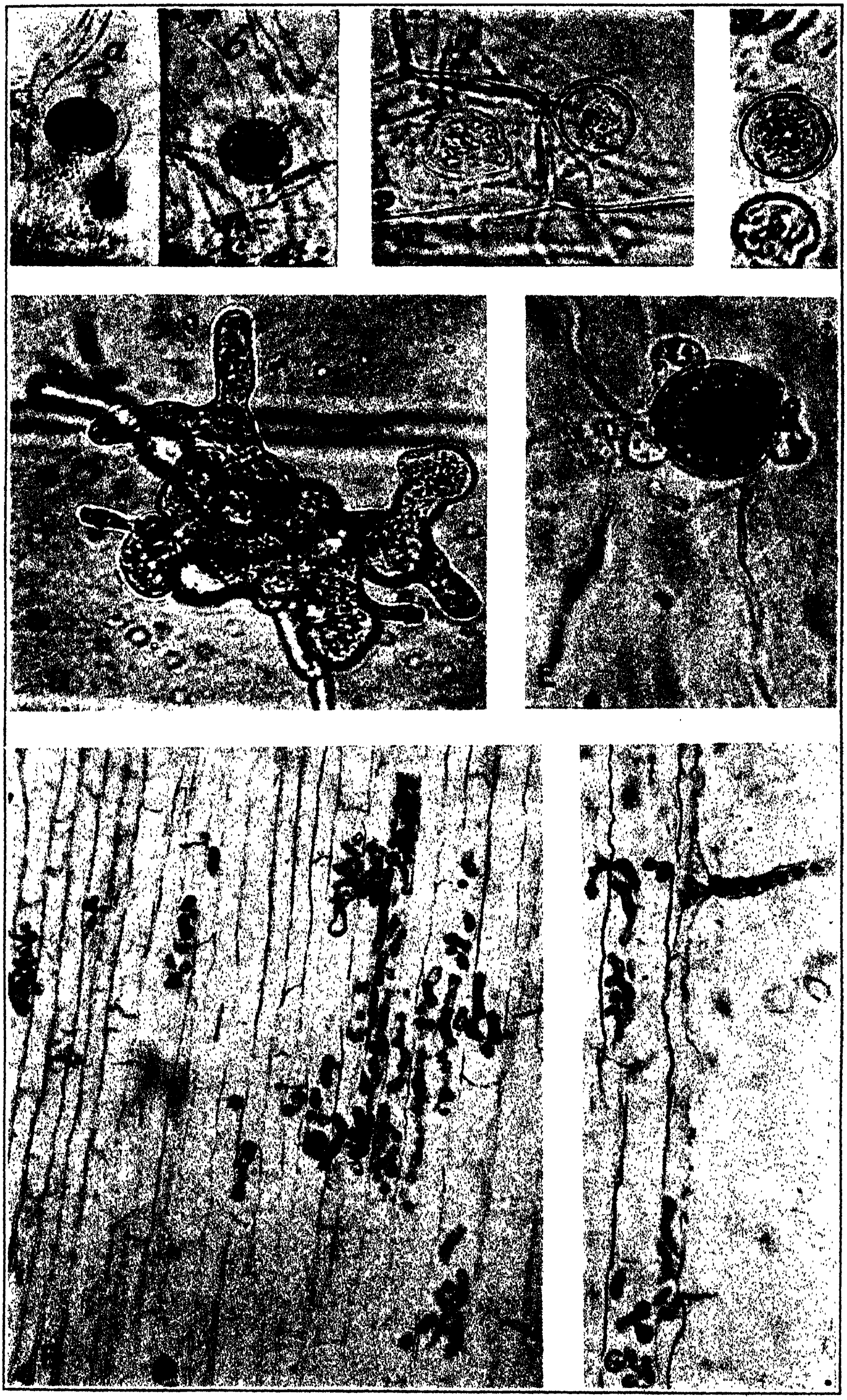
C.—Oospore formed on soil-decoction agar near the rootlet of a sterile corn seedling. Oospores were not found on the agar far from the roots of the seedlings. Grown in refrigerator.

D.—Lobulate-sporangium-grown-on water agar at room temperature. Culture 8 days old. Highly magnified.

E.—Oogonium and antheridia from a 10-day-old culture grown in the refrigerator on grated-carrot agar. × 550.

F.—Lobulate sporangia within the cortex of a corn seedling grown in wet soil at 16° C. × 200.

G.—Lobulate sporangium in a root hair of a corn seedling grown in wet soil at 16° C. × 200.



(For explanation of legend see p. 444)

CAUSAL ORGANISM

CULTURAL CHARACTERISTICS

Isolations were made by plating diseased tissues on water agar after carefully washing them in sterile water. A disinfectant was not used, as Pythium is sensitive to mercuric chloride and acids. The fungus grows rapidly and usually within two or three days grows away from any bacteria or other fungi that may be present, and therefore can be transferred readily. On water agar or on soil-decoction agar the mycelial growth is so delicate that it is scarcely visible unless examined by transmitted light. Oatmeal, potato-dextrose, and corn-meal agars favor a more dense, white mycelial growth. In water at room temperature complex masses of lobulate sporangia, called presporangia by Edson (*4*), form within a day or two along hyphae growing out of infected tissue. They may also be obtained within a week along hyphae on soft agars as poor in food materials as water agar or soil-decoction agar. (Pl. 1, D.) On these agars no conidia, oogonia, or antheridia have been found. They are numerous, however, at low temperatures in agar in which there is a plentiful supply of bits of plant tissue, such as grated carrots or string beans. (Pl. 1, E.) The conidia and oogonia are very similar in appearance during the early stages of their development. Later, under certain conditions, the conidia send out one or more germ tubes and the oogonia have one or more club-shaped antheridia applied to them. On the vegetable agars used, fertilization evidently is not of common occurrence, as comparatively few oospores are found. Likewise, in these media, typical lobulate sporangia are seldom observed, although thickenings of portions of the hyphae occur.

All three types of fruiting bodies have been obtained, however, within sterile roots of germinating corn seedlings. (Pl. 1.) For the most part they have been obtained from corn roots growing on inoculated soil-decoction agar in Petri dishes at relatively low temperatures (10° to 16° C.). Zoospores have been obtained within a few hours from corn roots containing lobulate sporangia by placing the roots in water in a Petri dish kept at room temperature. (Pl. 1, A.)

TEMPERATURE RELATIONS

The temperature range of this fungus in culture is wide. Two separate series of duplicate plates of potato-dextrose agar were inoculated in the center with squares, of as nearly equal size as possible, of a young agar culture and were incubated for three days at different temperatures ranging from 4° to 42° C. Mycelium, visible with a microscope, developed within 48 hours at 4° and at 40°, but an exposure of 72 hours at 42° killed the fungus. Maximum growth on this medium, as indicated by the diameters of the colonies, occurred between 30° and 36°.

The temperature at which the culture is grown, as well as the medium upon which it is grown, affects fruiting. Numerous lobulate sporangia appear within a few days on a favorable medium when grown at temperatures ranging from 20° to 30° C. These bodies are formed more slowly and in lesser numbers at the ordinary refrigerator temperatures (10° to 16°). On the other hand, oospore formation is favored by storage at these temperatures.

Subramaniam (*10*), who described *Pythium butleri* from India, found somewhat the same general conditions affecting its fruiting.

He states: "The development of sporangia and oogonia follows no regular sequence. When cultures are kept in a cool incubator at 21° C., oospores appear first and later on sporangia. At 30° C. sporangia appear first."

TAXONOMY

As stated by the writers in an earlier publication (*8*), this Pythium on corn is somewhat closely related to *Pythium butleri* Subr. on sugar cane, as described by Carpenter (*1*), who considered this species synonymous with *Rheosporangium aphanidermatum* Edson (*4*). Since then Fitzpatrick (*5*) has transferred this species to the genus Pythium, making the binomial *P. aphanidermatum* (Eds.) Fitzpatrick, inasmuch as Edson's work antedated that of Subramaniam.

Drechsler (*3*) reported *Pythium aphanidermatum* as the causal organism of cottony leak of cucumbers. He added that "a species not yet identified, provided with lobulate sporangia and hence closely related to but not identical with *P. aphanidermatum*, which was isolated from diseased corn roots, has shown no evidence of pathogenicity on cucumber fruit." The writers also have found cucumber fruit not an especially favorable medium for the growth of their corn Pythium. The fungus has proved mildly pathogenic to cucumbers, however, in a few wound inoculations.

Recently Valleau and his associates (*12*) report finding numerous oospores of the Pythium type in rotting corn roots. They suggest that "corn root rot, other than seedling blights known to be caused by certain seed-borne organisms, is caused by a fungus similar to the fungus causing the cane root rot."

The corn Pythium resembles the fungus reported by Carpenter as causing a root rot of sugar cane, in that it produces lobulate sporangia within the cortex of the roots and in culture. These are comparable to those described for the sugar-cane fungus. Likewise the measurements of the smooth oogonia and oospores correspond rather closely (Table 1) to those given by Carpenter.

TABLE 1.—*A comparison of certain characters of Rheosporangium aphanidermaum (4), Pythium butleri (10), the cane Pythium (1), and P. arrhenomanes*

[Measurements in microns]

Fungus	Zoospores	Oogonia		Oospores	
		Range	Average	Range	Average
R. aphanidermatum	12×7.5	22–27			17–19
P. butleri	8–12×6–8	24–33	26	13.5–25.3	21
Cane Pythium	10–12×7.5–9.5	[a] 24–35	29	21–28	24
		[b] 20–26		18–24	25
		[c] 21–30		20–25	22
P. arrhenomanes		24–35	29.9	21–28	24.4

[a] Culture. [b] Cane root from field. [c] Cane root in water culture.

Drechsler, who is making a special study of this group, has kindly examined cultures from the writers' material and finds that, while the fungus somewhat resembles *Pythium aphanidermatum* and certain related species, it differs distinctly. In an article by Drechsler, now in press, the parasite is being described as *P. arrhenomanes*.[4]

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[4] This paper by Drechsler is now published. DRECHSLER, C. PYTHIUM ARRHENOMANES N. SP., A PARASITE CAUSING MAIZE ROOT ROT. Phytopathology 18: 873–875. 1928

EXPERIMENTAL STUDIES

GREENHOUSE EXPERIMENTS

Experiments dealing with the effect of soil temperature and soil moisture on the occurrence of the disease were conducted in the Wisconsin soil-temperature control tanks in the greenhouses of the University of Wisconsin during the winters of 1923–24 and 1924–25. The temperatures used ranged from 12° to 32° C., at approximately 4° intervals. Experiments in the 32° tanks were discontinued during the second winter because of the scarcity of available tanks and because the checks and inoculated seedlings were equally poor when grown at such a high temperature under glass. Steam-sterilized soil was used throughout the experiments. Soil moisture was controlled by weighing the cans at intervals and adding the quantity of water necessary to maintain a constant weight. Different methods of watering were tried, but none was devised that could insure a uniform moisture distribution throughout the can, especially in the drier soils. When only one moisture was used in an experiment the soil was made up to a good growing moisture. When two moistures were employed the wet soil contained as much water as could be added and the soil screened and handled without packing—that is, about 70 per cent of saturation. The dry soils ranged from 45 to 55 per cent of saturation at the beginning of the experiments. In three series the inoculum was added to the soil some days previous to planting, and in the others small squares of water-agar or soil-decoction agar culture were placed near each seed at the time of planting. The control plants were grown in the sterilized soil without the addition of agar.

Table 2.—*Results of inoculating inbred strains of yellow dent corn with Pythium arrhenomanes at the time of planting in the greenhouse, Madison, Wis., 1923–1925*

Experiment No.	Date	Strain No.	Soil moisture	Inoculum used	Number of kernels germinating at— 12° C	16° C	20° C	24° C	28° C	Reduction in germination following inoculation	Number of plants standing at end of experiment at— 12° C	16° C	20° C	24° C	28° C	Reduction in stand following inoculation[a]	Mean length of tops at— 16° C	20° C	24° C	28° C	Reduction following inoculation	Mean dry weight of tops at— 16° C	20° C	24° C	28° C	Reduction following inoculation
										P. ct.						*P. ct.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*P. ct.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. ct.*
5	Oct. 10 to Dec. 12	Hy-826-1-5-1.	Wet	None	15	16	16	16	16		11	14	16	13	16		10.7	17.4	28.7	27.7		0.535	0.954	2.565	2.715	
				Pythium	14	16	16	16	15	2.53	13	16	15	11	14	1.43	8.7	17.0	25.4	27.5	6.98	.262	.669	1.575	2.905	20.06
			Dry	None	8	14	15	13	15		8	11	15	8	13		7.5	13.8	16.7	15.1		.280	.378	.275	.695	
				Pythium	6	13	16	14	15	1.54	6	11	16	10	14	[a]+3.64	4.7	10.7	13.4	17.0	13.75	.151	.232	.300	.700	15.05
		Hy-826-1-6-1.	Wet	None	12	12	15	16	14		12	11	14	15	11		8.5	14.1	22.3	25.8		.446	.743	1.845	2.890	
				Pythium	15	12	15	15	16	[a]+5.80	8	4	14	14	13	15.87	5.1	12.9	22.0	21.9	12.45	.158	.804	1.945	1.725	21.81
			Dry	None	8	15	8	11	7		8	[b]8	8	10	6		6.0	9.9	12.7	11.7		.164	.412	.427	.437	
				Pythium	12	11	10	10	11	[a]+10.20	11	11	10	10	11	[a]+32.50	5.2	7.9	16.2	12.8	[a]+4.47	.131	.243	.650	.342	5.14
7 and 8	Feb. 9 to Apr. 24[c] and Mar. 7 to Apr. 24[d]	A-1-1-2-R-1-2-3.	Wet	None	16	16	14	16	16		16	16	14	15	15		15.7	24.2	38.5	30.5						
				Pythium	0	0	15	14	15	43.59	0	0	14	14	15	43.42	0	13.6	33.4	29.3	29.94					
			Dry	None	15	14	15	13	16		15	14	15	11	15		12.9	21.6	38.0	26.2						
				Pythium	0	14	16	16	16	15.07	0	14	16	14	16	14.29	9.6	16.1	31.5	27.0	14.69					
		A-1-1-4-1-8-1-5.	Wet	None	10	16	16	16	14		10	16	16	16	14		9.2	21.6	35.5	26.1						
				Pythium	1	1	11	13	11	48.61	1	1	11	13	10	50.00	4.0	10.1	25.7	22.8	32.25					
			Dry	None	11	13	16	15	15		11	13	16	14	15		7.7	19.4	33.5	24.4						
				Pythium	0	2	14	16	15	32.86	0	2	14	15	13	36.23	7.5	13.3	26.8	22.5	17.53					
9	May 4 to May 29	B-1-1-3-R-10-1-12.	Wet	None		15	16	16	16			15	11	15	16		10.9	22.3	40.5	50.2			.337	.720	.750	
				Pythium		15	15	16	16	1.59		13	15	16	16	[a]+5.26	7.0	17.8	32.6	40.7	20.82		.206	.318	.437	46.82
			Dry	None		16	16	16	16			16	16	16	14		10.7	21.8	38.9	45.8			.312	.637	.715	
				Pythium		16	16	16	16	0		14	16	16	16	0	5.4	20.1	36.4	45.0	8.79		.243	.456	.543	25.36
		A-1-1-1-R-4-2-4.	Wet	None		16	16	16	14			16	16	15	13		11.8	18.2	35.2	47.2			.250	.660	.669	
				Pythium		13	15	16	16	3.23		13	15	16	16	0	7.7	15.3	31.0	42.8	13.88		.180	.450	.493	28.88
			Dry	None		16	16	16	16			16	16	15	16		10.4	19.1	34.0	45.1			.238	.526	.556	
				Pythium		16	16	16	16	0		15	15	16	16	1.59	8.0	16.7	34.2	42.1	7.00		.206	.512	.518	6.36
		G-146	Wet	None		[b]16	16	16	16			15	16	15	14		22.03	32.5	45.5	43.71		.093	.206	.333	.213	
				Pythium		12	16	[b]16	16	6.25		5	15	11	13	26.67	17.80	24.4	44.5	52.07	3.46	.060	.140	.318	.361	[a]+4.02
			Dry	None		15	15	16	16			15	15	16	16		17.40	26.26	41.4	44.30		.073	.106	.218	.231	
				Pythium		14	15	16	16	1.61		10	15	16	15	9.68	15.35	26.20	37.2	43.80	5.26	.050	.125	.187	.233	5.25
10	Oct. 24 to Dec. 10	A-1-1-2-R-1-2-3.	Wet	None		16	15	16	[b]16			12	15	15	15		22.83	34.26	50.66	56.50		.077	.213	.506	.600	
				Pythium		10	16	[b]16	16	7.94		3	13	13	15	22.81	12.66	29.61	51.50	53.80	10.16	.016	.161	.484	.546	13.54
			Dry	None		[b]16	16	16	16			12	15	15	15		18.41	32.73	50.73	48.60		.066	.166	.368	.366	
				Pythium		[b]16	15	16	15	3.13		15	15	14	14	[a]+1.75	16.20	35.00	45.79	44.90	5.70	.065	.180	.342	.357	2.28
Per cent reduction following inoculation					49.5	25.2	1.66	0.82	[a]+.84		57.1	33.2	2.14	2.2	[a]+1.34		33.43	17.9	9.81	4.03		48.5	21.5	17.0	15.5	

[a] + denotes increase.

[b] 1 can with 4 plants discarded.

[c] 12° C.

[d] 16° to 28° C.

TABLE 3.—*Summary of the results of inoculating inbred strains of yellow dent corn with Pythium arrhenomanes at the time of planting in the greenhouse, Madison, Wis., 1923–1925*

Temperature (°C.)	Kernels germinating			Plants standing at end of experiment			Mean length of tops			Mean dry weight of tops		
	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (cm.)	Inoculated (cm.)	Reduction following inoculation (per cent)	Control (gram)	Inoculated (gram)	Reduction following inoculation (per cent)
12	95	48	49.5	91	39	57.1						
16	242	181	25.2	220	147	33.2	12.7	8.4	33.4	0.216	0.111	48.5
20	241	237	1.7	234	229	2.1	21.8	17.9	17.9	.359	.282	21.5
24	244	242	.8	224	219	2.2	35.2	31.7	9.8	.756	.628	17.0
28	239	241	[a]+.8	224	227	[a]+1.3	35.6	34.1	4.0	.903	.763	15.5

[a] + denotes increase.

The data obtained in these experiments from the use of the inbred strains are given in Table 2, summarized in Table 3, and presented graphically in Figure 1. Those obtained from the use of open-pollinated yellow dent corn are given in Table 4 and shown graphically in Figure 2.

TABLE 4.—*Results of inoculating open-pollinated yellow dent corn with Pythium arrhenomanes at time of planting in the greenhouse, Madison, Wis., 1923–24*

Temperature (°C.)	Kernels germinating			Plants standing at end of experiment			Mean length of tops			Mean dry weight of tops		
	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (cm.)	Inoculated (cm.)	Reduction following inoculation (per cent)	Control (gram)	Inoculated (gram)	Reduction following inoculation (per cent)
16	28	22	21.4	27	20	25.9	23.39	18	23.0	1.05	0.52	50.5
20	29	28	[a]3.4	25	27	[a][b]+8.0	29.50	26.23	[a]11.1	2.07	1.31	[a]36.7
24	28	29	[b]+3.6	27	28	[b]+3.7	29.66	24.86	16.2	2.12	1.32	37.7
28	30	30	0	24	24	0	27.30	24.29	11.0	1.93	1.34	30.6
32	29	30	[b]+3.4	19	20	[b]+5.3	23.46	24.20	[b]+3.2	1.12	1.17	[b]+4.5

[a] The drop in these percentages at 20° was due in some measure to the presence of an electric light hung directly above the 20° tank and kept lighted throughout the night.

[b] + denotes increase.

At the higher temperatures germination was not materially affected following inoculation with *Pythium arrhenomanes*. Soil temperatures as low as 12° C. caused an uneven stand in both the uninoculated and the inoculated series, indicating an extremely unfavorable condition for the corn seedlings. At 16° the reduction in germination following inoculation averaged 25.2 per cent in the inbred strains (Tables 2 and 3) and 21.4 per cent in the open-pollinated corn (Table 4). In the inbred strains the reduction ranged from zero in strains B–1–1–3–R–10–1–12 and A–1–1–1–R–4–2–4 to 100 per cent in strain A–1–1–2–R–1–2–3. (Fig. 3.) Kernels planted in wet soil usually were more severely attacked than those in dry soil. At 20°

the average reduction was only 1.66 per cent (Tables 2 and 3) and the greatest reduction was 31.3 per cent.

The lack of uniformity in germination occurring in the dry soil may be ascribed in part at least to the difficulty of securing an even distribution of moisture throughout the dry soil after watering began.

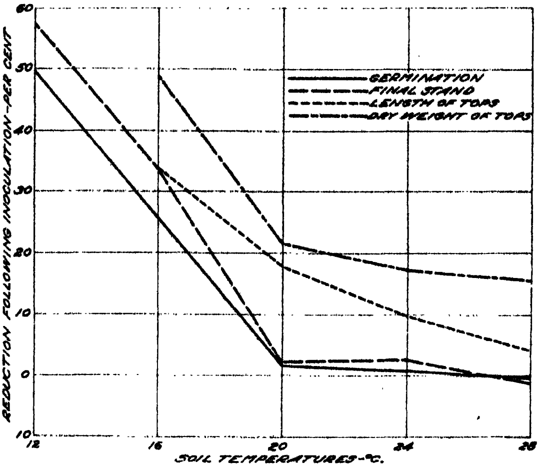

FIG. 1.—Reductions in germination of seed, in final stand, and in length and dry weight of tops of corn plants grown at different soil temperatures from inbred seed, inoculated with *Pythium arrhenomanes* at the time of planting. (Data in Table 3)

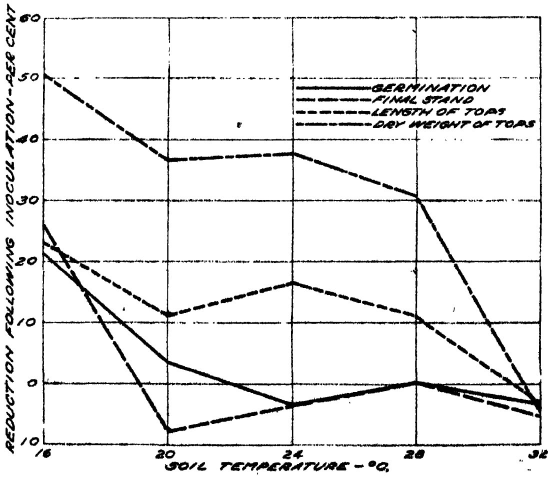

FIG. 2.—Reductions in germination of seed, in final stand, and in length and dry weight of tops of corn plants grown at different soil temperatures from open-pollinated seed, inoculated with *Pythium arrhenomanes* at the time of planting. (Data in Table 4)

Blighting of seedlings after emergence further reduced the stand, and low soil temperatures favored this manifestation of the disease.

A reduction in plant height and dry weight of tops followed inoculation with *Pythium arrhenomanes*. The highest percentage of reduction both in height and in dry weight of tops occurred at the lower temperatures. High soil moisture also tended to favor this manifestation of the disease. These plants showed no indication of the disease except the dwarfing of the aboveground parts. The tops were of good color and unwilted. Upon removal from the soil, however, the root systems were found to be smaller and in most cases partially destroyed. The root rot is not confined to those strains most susceptible to seedling blight. Strain B–1–1–3–R–10–1–12, with a good record for germination, was reduced in height and in dry weight of tops following inoculation (fig. 4), although, as pointed out above, germination was not reduced materially. *P. arrhenomanes* was observed in or isolated from diseased roots at all temperatures.

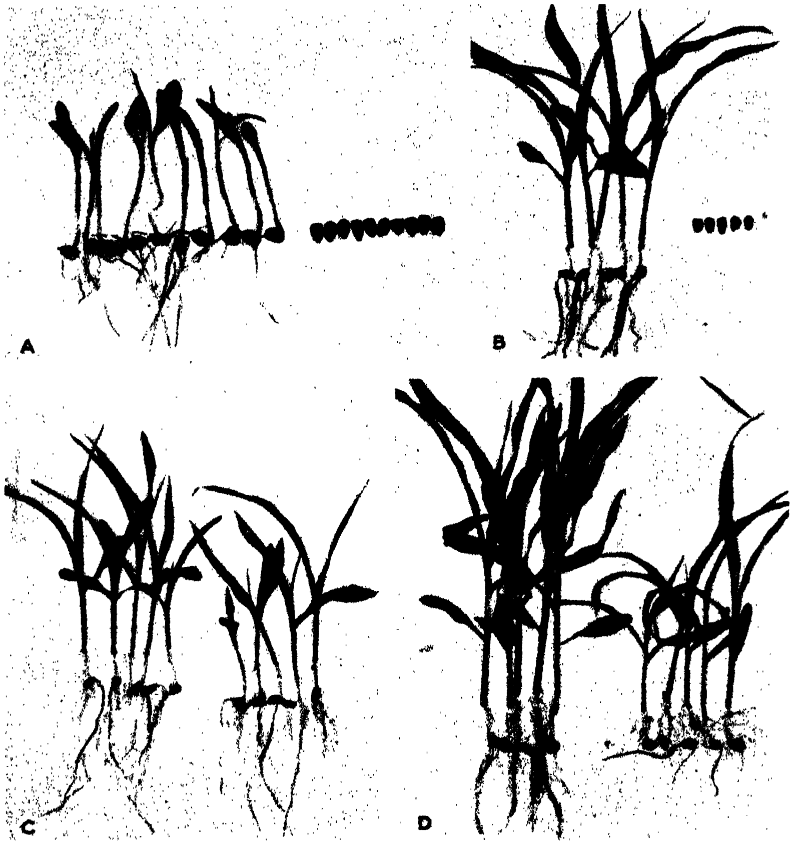

FIG. 3.—Corn seedlings of inbred strain A–1–1–2–R–1–2–3, uninoculated and inoculated with *Pythiumar rhenomanes* at time of planting, grown at different soil temperatures and in wet and dry soil. In each case the seedlings from uninoculated seed are grouped at the left and those from inoculated seed at the right. A, Grown in wet soil at 12° C.; B, grown in wet soil at 16°; C, grown in dry soil at 16°; D, grown in wet soil at 20°

FIELD EXPERIMENTS

In addition to the greenhouse inoculation experiments, field inoculations were made near Bloomington, Ill., in 1924, 1925, and 1926. In these field experiments the weather conditions were studied in connection with the development of the disease and the relative susceptibility of the different strains of corn.

WEATHER CONDITIONS AND EXPERIMENTS IN 1924

The spring of 1924 was cool, with a well-distributed rainfall during the period of seedling growth of the corn plants. The mean air temperature at Bloomington, Ill., for the month of May was 6.4° F. below the normal (*11*). For June the departure from normal was −3.4. Precipitation in May was 3.2 inches, 1.36 inches below

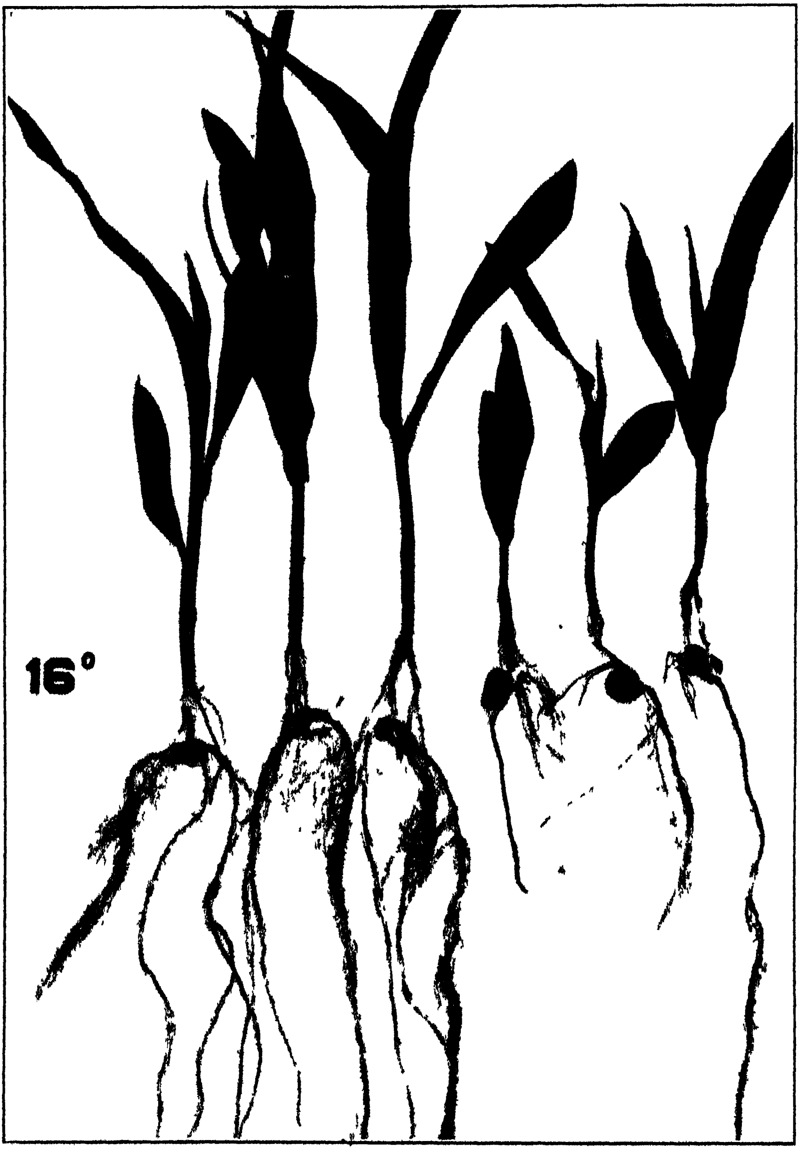

FIG. 4.—Corn seedlings of inbred strain B-1 1-3-R-10-1-12. The three at the left were uninoculated; the three at the right were inoculated with *Pythium arrhenomanes* at the time of planting. Grown in moderately wet soil at 16° C.

the average. In June, however, a 2.5-inch rainfall on the 22d brought the total for the month to 7.81 inches, an excess of 4.2 inches. The field temperatures during the period of seedling development of the corn plant in 1924 (fig. 5) were comparable, in general, to the conditions in the low-temperature tanks in the greenhouse. The mean air and soil temperatures were obtained for each day by adding the maximum and minimum temperatures and dividing by 2.

Twenty-three inbred strains and one open-pollinated strain of yellow dent corn were used in the field experiments in 1924. The corn was checked 3½ feet apart with three kernels in each hill. Five rows of each strain were planted in each of two positions in the field. The first five hills of each row, which were left uninoculated, served as a control; the next five hills in each of the five rows were inoculated with sand cultures[5] of *Pythium arrhenomanes*. The following five hills served as a control. The succeeding five hills in each row were inoculated with conidial suspensions of *Gibberella saubinetii;* the next five hills were controls, after which the Pythium inoculation and controls were repeated. The results presented, therefore, represent a mean of the four 25-hill inoculated plots for each selfed line compared with the eight adjacent 25-hill control plots. The inoculum used for Pythium consisted of a small mass of mycelium growing in corn meal and white sand, which was placed in each hill just before the kernels were dropped into it. The control plots were planted without the addition of the sterilized sand and corn meal, as insufficient corn meal was used in the medium to affect materially the seedling growth of the control plants.

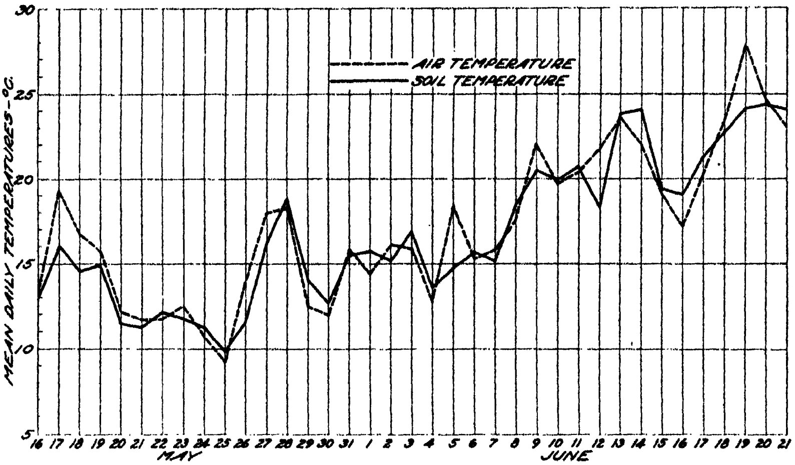

FIG. 5.—Daily mean air temperatures and mean of daily soil temperatures taken at 6 a. m. and 5.30 p. m. at Bloomington, Ill.

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[5] The corn-meal-sand medium was prepared by washing 1 volume of quartz sand in distilled water, draining off the water but not drying the sand, and adding to this 1 volume of yellow corn meal and 1 to 1½ volumes of water. After a thorough stirring the mixture was steamed for 1 hour, stirred again, and allowed to cool. It was then broken into small pieces, placed in flasks, and autoclaved for 45 minutes at a pressure of 10 to 12 pounds on 2 successive days. Rapid growth of the fungus is obtained if the flasks are inoculated as soon as prepared. Cultures used in these experiments were usually about 10 days old.

TABLE 5.—*Results of inoculating open-pollinated and inbred strains of yellow dent corn with Pythium arrhenomanes planted near Bloomington, Ill., May 16, 1924*

Strains inoculated	Inoculum used	Number of replications	Mean field stand (per cent)	Mean yield per plant (pounds)	Effect of inoculation on plant yield			Acre yield		Effect of inoculation on acre yield					
										Total			Sound corn		
					Increase (pounds)	Decrease (pounds)	Odds	Total (bushels)	Sound corn (bushels)	Increase (bushels)	Decrease (bushels)	Odds	Increase (bushels)	Decrease (bushels)	Odds
Open-pollinated:															
Nearly disease-free seed (176A)	None	5	79.7	0.864				88.0	73.8						
	Pythium	5	64.0	.819		0.045	8:1	66.6	58.1		21.4	>9999:1		15.7	462:1
Diplodia-infected seed (176A)	None	5	48.5	.908				57.1	45.6						
	Pythium	5	34.9	.941	0.033		1:1	40.7	31.9		16.4	70:1		13.7	188:1
Inbred:															
A-1-1-1-R-4-2	None	4	54.6	.506				34.3	24.2						
	Pythium	4	38.7	.441		.065	4999:1	19.3	9.8		15.0	>9999:1		14.4	>9999:1
A-1-1-2-R-1-1-2	None	4	57.0	.443				30.2	26.5						
	Pythium	4	28.7	.500	.057		20:1	16.2	14.8		14.0	516:1		11.7	26:1
A-1-1-3-R-3-1	None	4	45.4	.357				22.3	17.4						
	Pythium	4	20.7	.474	.117		78:1	13.1	9.9		9.2	41:1		7.5	40:1
A-1-1-4-1-8-1	None	4	33.4	.490				20.9	15.3						
	Pythium	4	28.0	.476		.014	322:1	17.1	9.4		3.8	113:1		5.9	322:1
A-1-1-4-1-9-5	None	4	37.4	.324				14.6	8.6						
	Pythium	4	32.0	.375	.051		4:1	14.4	9.2		.2	1:1	0.6		10:1
B-1-1-1-1-8-1	None	4	50.7	.280				15.1	5.9						
	Pythium	4	58.7	.244		.036	55:1	15.3	6.4	0.2			.5		4:1
B-1-1-3-R-7-3	None	4	60.0	.563				40.4	24.9						
	Pythium	4	24.0	.514		.049	1666:1	14.8	8.0		25.6	>9999:1		16.9	1082:1
B-1-1-3-R-10-2	None	4	62.0	.526				43.1	32.8						
	Pythium	4	57.3	.523		.003	1:1	39.6	29.0		3.5	4:1		3.8	4:1
K-4-3-1	None	4	60.0	.400				37.0	27.0						
	Pythium	4	38.7	.578	.178		>9999:1	26.8	17.6		10.2	55:1		9.4	35:1

G-8-8-1	None	4	72.0	.502				42.8	33.8						
	Pythium	4	51.7	.384		.118	>9999:1	21.5	18.7		18.3	>9999:1		15.1	>9999:1
L-1-1-1-1	None	4	71.3	.676				47.3	41.7						
	Pythium	4	47.4	.548		.128	160:1	28.5	20.4		20.8	>9999:1		21.3	>9999:1
ID-3-1-1-1-1-1	None	1	72.0	.515				46.1	27.4						
	Pythium	1	40.0	.377		.138		18.6	9.1		27.5			18.3	
228-4-8-2-1	None	1	74.7	.434				36.1	19.9						
	Pythium	1	42.7	.463	.029			22.1	12.0		14.0			7.9	
W-2-1-3	None	1	72.0	.315				23.6	7.3						
	Pythium	1	53.3	.188		.127		11.6	2.7		15.0			4.6	
W-10-2-2	None	1	74.7	.625				59.2	47.3						
	Pythium	1	58.7	.541		.084		40.2	33.4		19.0			13.9	
M-3-3-1	None	1	65.3	.527				43.3	30.7						
	Pythium	1	53.3	.495		.032		33.2	17.6		10.1			13.1	
HY-823-1-5-1	None	4	72.7	.594				46.7	35.6						
	Pythium	4	60.0	.610	.016		3:1	39.6	30.3		7.1	7:1		5.3	4:1
HY-826-1-6-1	None	4	44.8	.405				18.7	14.9						
	Pythium	4	38.0	.483	.078		38:1	19.4	16.4	.7		21:1	1.5		6:1
A-1-1-2-R-3-2	None	4	62.7	.478				31.1	23.8						
	Pythium	4	41.3	.590	.112		26:1	25.8	23.3		5.3	2069:1		.5	3:
B-1-1-1-1-7-2	None	4	47.3	.523				29.0	23.3						
	Pythium	4	28.0	.500		.023	2:1	14.8	12.7		14.2	348:1		10.6	645:1
G-4-2-1	None	4	71.4	.311				25.6	13.3						
	Pythium	4	38.5	.443	.132		22:1	19.8	10.2		5.8	148:1		3.1	5:1
G-4-4-1	None	4	53.3	.323				20.0	10.0						
	Pythium	4	21.3	.372	.049		221:1	8.9	4.6		11.1	405:1		5.4	2333:1
B-1-1-1-R-8-1-2	None	8	66.0	.510				37.1	25.1						
	Pythium	8	45.0	.560	.050		65:1	25.9	16.8		11.2	1523:1		8.3	59:1
Mean (of the 23 inbred strains)	None		60.0	.462				33.37	23.33						
	Pythium		41.3	.464				22.07	14.88						

The corn was planted on May 16, when the soil temperature was about 56° F. (13.5° C.). The soil was fairly dry. On May 18 there was a 0.56-inch rainfall, followed by a gradual decline in temperature during the following week. The corn germinated, therefore, in a cool, fairly moist soil, comparable to the conditions in the low-temperature tanks in the greenhouse. The reduction in stand and comparative yields are given in Table 5.

Reduction in stand, as compared with the controls, occurred in all cases with one exception. In the control plots a mean stand of 60 per cent was obtained in the 23 inbred strains. The mean stand in the same strains inoculated with *Pythium arrhenomanes* was 41.3 per cent. The reduction in stand due to inoculation was 31 per cent. In the nearly disease-free and Diplodia-infected open-pollinated corn the reductions in stand chargeable to the inoculation were 19.7 and 28 per cent, respectively. When yields were considered, the reduction in stand was more or less counterbalanced by an increased yield per plant in certain of the corn strains. In such cases the Pythium injury evidently took the form of a seedling blight rather than a root rot of the developing plant. In other strains the yield per plant was decreased notwithstanding the reduction in stand. Total yield was generally reduced, the reduction being greater with certain strains of corn than with others. (Figs. 6 and 7.)

The maximum seedling injury following inoculation with *Pythium arrhenomanes* occurred in strain B–1–1–3–R–7–3, where the stand was reduced 60 per cent. Parallel inoculations with *Gibberella saubinetii* caused the maximum reduction in stand of 88.8 per cent in the case of strain K–4–3–1. The mean reduction of sound corn per acre for all strains following inoculation with *P. arrhenomanes* was 36.2 per cent, as compared with 53.3 per cent in the similar Gibberella inoculations.

In the 23 inbred strains the mean yield per plant in the uninoculated controls was 0.462 pound, while that in the series inoculated with *Pythium arrhenomanes* was 0.464 pound per plant. In a parallel series in which *Gibberella saubinetii* was used the mean yield per plant in the uninoculated controls was 0.468 pound and that in the inoculated series 0.478 pound per plant.

WEATHER CONDITIONS AND EXPERIMENTS IN 1925

Weather conditions in the spring of 1925 did not closely parallel those of 1924. The mean air temperature for the entire month of May was 59.2° F., 3.7° below normal. It was the first nine days of the month, however, which were decidedly below the average. The average for the second half of the month was practically normal. Precipitation for the entire month was very low. During the first two weeks of June the mean maximum air temperature rose to 89°. Precipitation again was below normal. The months of May and June, 1925 (figs. 8 and 9), were characterized by a higher mean temperature with wider fluctuations and with less precipitation than in 1924.

In 1925 five inbred lines of corn were planted on two dates. Each line was inoculated at the time of planting with *Pythium arrhenomanes* and *Gibberella saubinetii* in parallel series, accompanied by uninoculated rows which served as controls. The first planting was made on May 12 in a cool, very dry soil. There was a light rain on May

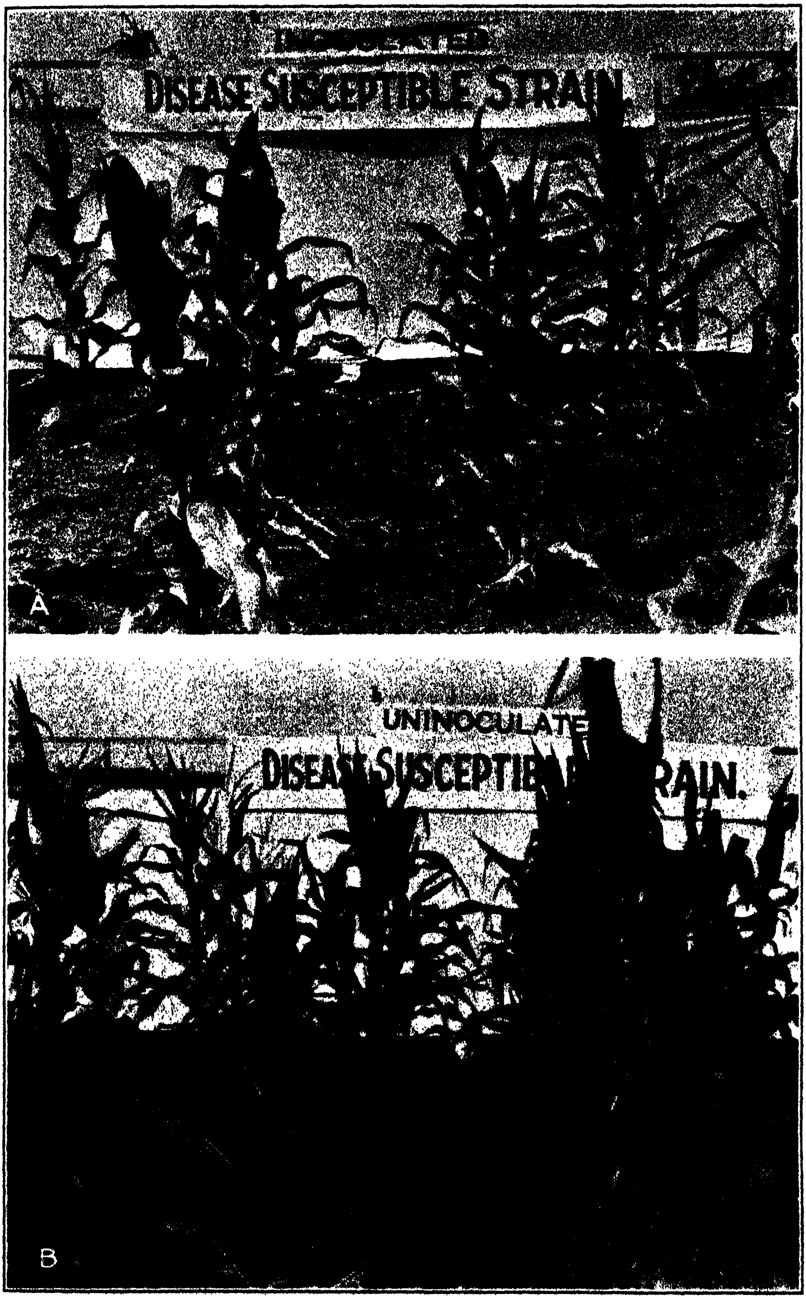

FIG. 6.—Yellow dent corn plants of a strain relatively susceptible to *Pythium arrhenomanes*, grown at Bloomington, Ill., in 1924. A, Inoculated with *P. arrhenomanes* at time of planting; B, uninoculated

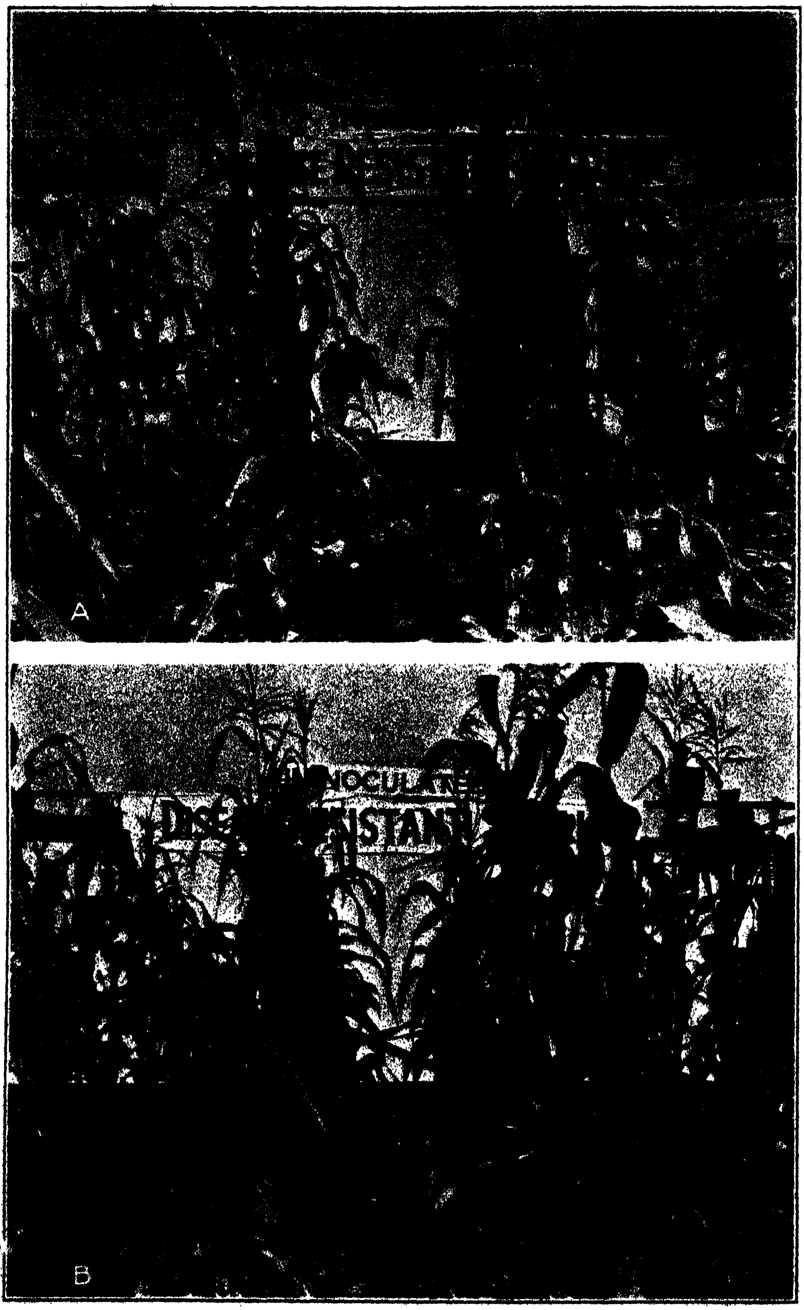

FIG. 7.—Yellow dent corn plants of a strain resistant to *Pythium arrhenomanes*, grown at Bloomington, Ill., in 1924. A, Inoculated with *P. arrhenomanes* at time of planting; B, uninoculated

16, sufficient to permit the germination of the seed, but still the soil was dry. The second planting was made on June 4 in a warm, dry soil. This season, then, offered temperature conditions representing low and high soil temperatures, but only low soil moisture in both cases. As a result, the stand was much more irregular, as some kernels did not have sufficient moisture for germination. The results are given in Table 6.

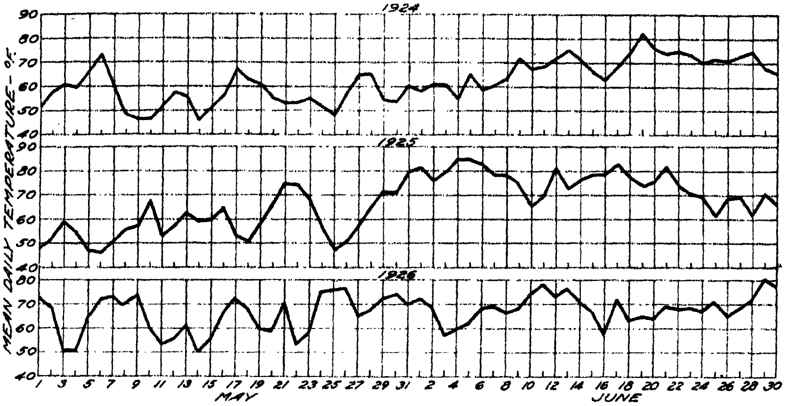

FIG. 8.—Daily mean air temperatures at Bloomington, Ill., during May and June, 1924, 1925, and 1926, respectively

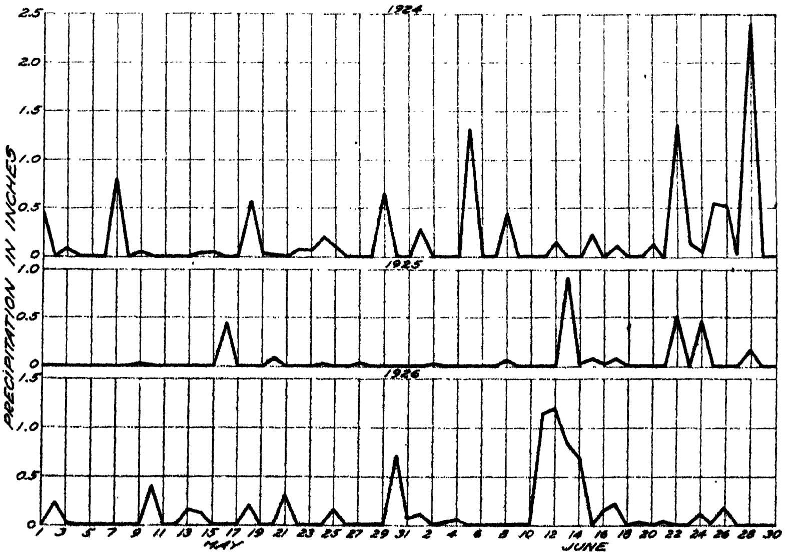

Fig. 9.—Precipitation at Bloomington, Ill., during May and June, 1924, 1925, and 1926, respectively

For the June 4 planting of strain A-1-1-2-R-1-1-9 no results with Pythium are presented. At that point in the field pocket gophers and field mice disturbed the stand to such an extent that it was deemed unwise to include the data in the table.

TABLE 6.—*Results of inoculating five inbred strains of yellow dent corn with Pythium arrhenomanes and Gibberella saubinetii, planted near Bloomington, Ill., 1925*

Strains	Date of planting	Inoculum used	Number of replications	Field stand (per cent)	Mean yield per plant (pounds)	Mean yield per acre (bushels)	Reduction in acre yield following inoculation		
							Bushels	Per cent	Odds
A-1-1-2-R-1-1-9	May 12	None	4	37.4	0.319	16.1			
		Pythium	4	16.0	.536	12.9	3.2	19.9	13:1
	do	None	7	64.0	.583	18.7			
		Gibberella	7	39.7	.543	10.9	7.8	41.7	>9999:1
	June 4	None	4	75.3	.329	37.9			
		Gibberella	4	62.0	.394	33.1	4.8	12.7	19:1
A-1-1-2-R-1-2-3	May 12	None	8	30.5	.251	12.6			
		Pythium	8	4.3	.275	1.7	10.9	86.5	>9999:1
	June 4	None	4	67.7	.229	23.1			
		Pythium	4	27.3	.296	12.2	10.9	47.2	434:1
	May 12	None	8	33.2	.258	13.2			
		Gibberella	8	4.7	.358	2.3	10.9	82.6	>9999:1
	June 4	None	4	69.4	.221	22.6			
		Gibberella	4	71.3	.290	29.6	[a]+7.0	[a]+31.0	257:1
A-1-1-4-1-9-5-6	May 12	None	8	21.7	.205	6.9			
		Pythium	8	3.4	.098	.6	6.3	91.3	>9999:1
	June 4	None	4	56.4	.299	25.9			
		Pythium	4	21.3	.243	8.4	17.5	67.6	37:1
	May 12	None	8	22.2	.194	6.8			
		Gibberella	8	1.3	.000	.0	6.8	100.0	>9999:1
	June 4	None	4	62.4	.306	29.1			
		Gibberella	4	68.7	.296	30.8	[a]+1.7	[a]+5.8	3:1
A-1-1-4-1-8-1-5	May 12	None	8	10.0	.269	4.5			
		Pythium	8	2.0	.083	.5	4.0	88.9	>9999:1
	June 4	None	4	55.0	.347	29.0			
		Pythium	4	44.7	.291	20.3	8.7	30.0	35:1
	May 12	None	8	13.5	.212	5.0			
		Gibberella	8	.3	.050	.1	4.9	98.0	>9999:1
	June 4	None	4	58.4	.356	31.9			
		Gibberella	4	57.3	.359	31.3	.6	1.9	1:1
B-1-1-3-R-10-1-12	May 12	None	8	47.7	.553	39.4			
		Pythium	8	12.0	.482	9.2	30.2	76.6	>9999:1
	June 4	None	2	81.4	.411	50.9			
		Pythium	2	60.0	.496	45.3	5.6	11.0	7:1
	May 12	None	8	48.1	.544	38.7			
		Gibberella	8	33.4	.527	26.7	12.0	31.0	41:1
	June 4	None	4	72.3	.458	49.8			
		Gibberella	4	69.4	.458	47.3	2.5	5.0	2:1

[a] + denotes increase.

The stand of the five inbred strains in both plantings was reduced following inoculation with *Pythium arrhenomanes*. The stand of the earlier planting was reduced to a greater extent than that of the later planting. The mean yield per plant varied with the different strains of corn, but in every case the reduction was greater in the early planting.

Following the inoculations with *Pythium arrhenomanes*, reductions in stand were accompanied by increased yields per plant in both plantings of strains A-1-1-2-R-1-2-3, in the June 4 planting of B-1-1-3-R-10-1-12, and in the one planting of A-1-1-2-R-1-1-9. The other inoculations, both early and late, were followed by a reduced stand and a reduced yield per plant. Parallel inoculations with *Gibberella saubinetii* resulted in as great a reduction in stand in the first planting. In the later planted series, however, injury following the inoculations with *G. saubinetii* was slight, if any, whereas following inoculation with *P. arrhenomanes* there was an appreciable reduction in stand and acre yield.

WEATHER CONDITIONS AND EXPERIMENTS IN 1926

The mean temperature during May, 1926, was higher than that of the two preceding seasons, averaging 2.1° F. above normal. The rainfall (2.37 inches) was 1.96 inches below the average for May for the 36 years on record. June, on the other hand, was a relatively cool month, the mean temperature being 68°, 4° below the normal. Rainfall for the month totaled 4.76 inches, which was a fraction of an inch above the average.

The field experiments at Bloomington, Ill., in 1926 included inoculations of inbred strains of corn with *Pythium arrhenomanes*, as in the previous years, and of several crosses and back crosses of inbred strains. (Table 7.)

TABLE 7.—*Results of inoculating inbred strains and crosses of yellow dent corn with Pythium arrhenomanes, planted near Bloomington, Ill., 1926*

[Average of eight replications]

Strain or cross	Field stand in uninoculated plots (per cent)	Percentage increase (+) or decrease (−) in field stand following inoculation	Odds	Plant yield in uninoculated plots (pounds)	Percentage increase (+) or decrease (−) in plant yield following inoculation	Odds	Acre yield from uninoculated plots (bushels)	Percentage increase (+) or decrease (−) in acre yield following inoculation	Odds
Inbred: A-1-1-4-1-8-1-5	65.3	−4.0	3:1	0.321	−8.1	59:1	32.1	−12.8	40:1
Inbred: A-1-1-4-1-9-5-6	62.7	−2.2	2:1	.351	−8.8	66:1	32.8	−9.1	6:1
Cross, F₁: A-1-1-2-R-1-1-9×A-1-1-4-1-9-5-6	78.7	−3.4	2:1	.659	−.6	1:1	79.2	−5.9	6:1
Back cross: A-1-1-2-R-1-1-9×A-1-1-4-1-9-5-6×A-1-1-4-1-9-5-6	70.0	−14.3	103:1	.508	−7.7	11:1	53.8	−11.6	77:1
Back cross: A-1-1-2-R-1-1-9×A-1-1-4-1-8-1-5×A-1-1-4-1-8-1-5	81.3	−6.5	55:1	.543	−4.6	14:1	64.4	−10.4	103:1
Inbred: A-1-1-2-R-1-1-9	73.3	−5.5	8:1	.383	−1.0	1:1	40.2	−10.2	21:1
Cross, F₁: A-1-1-2-R-1-1-9×B-1-1-3-R-10-1-12	73.3	−10.9	52:1	.732	+4.0	28:1	79.8	−7.1	17:1
Cross, F₂: A-1-1-2-R-1-2-3×B-1-1-3-R-10-1-12	74.7	−23.3	932:1	.456	−1.5	1:1	50.8	−22.8	1249:1
Inbred: B-1-1-3-R-10-1-12	74.7	−25.0	382:1	.376	+4.8	------	41.4	−21.5	117:1
Cross, F₁: A-1-1-2-R-1-2-3-7-×B-1-1-3-R-10-1-12	76.0	−8.8	28:1	.760	+5.4	81:1	86.7	−2.3	3:1
Inbred: A-1-1-2-R-1-2-3-7	66.6	−11.9	59:1	.292	+4.5	2:1	27.7	−2.5	2:1
Cross, F₁: G-8-8-1×A-1-1-2-R-1-1-9	80.0	−1.6	2:1	.722	−3.9	9:1	81.5	−5.4	11:1

In no case did the stand or acre yield in the inoculated plots equal that in the uninoculated plots. The greatest reduction in stand, 25 per cent (odds 382:1), occurred in strain B–1–1–3–R–10–1–12. This maximum was closely followed by a 23.3 per cent reduction in stand (odds 932:1) in the F_2 cross of A–1–1–2–R–1–2–3×B–1–1–3–R–10–1–12. The percentage reduction in acre yield nearly equaled the reduction in stand in the two instances. Two inbred strains and two crosses gave an increased yield per plant following a reduction in stand.

Of the inbred strains, B-1-1-3-R-10-1-12 produced the highest yield per acre in the uninoculated plots. It showed, however, the greatest reduction in stand and acre yield following inoculation with *Pythium arrhenomanes*. On the other hand, strain A-1-1-2-R-1-2-3-7, with a poor stand and the lowest yield per acre, was reduced only 2.5 per cent (odds 2:1). The F_1 cross between these two strains resulted in the highest yield per acre in the uninoculated plots and only a 2.3 per cent reduction (odds 3:1) in acre yield following inoculation.

DISCUSSION

It is a matter of common observation that thinning the stand, up to a certain point, tends to increase the yield per plant. Kiesselbach (*9*), experimenting with Hogue Yellow Dent corn over a four-year period (1914–1917), found that 1 plant per hill (3,556 per acre) averaged 40.7 bushels per acre, or 0.64 pound per plant. Two plants per hill (7,112 per acre) yielded 49.4 bushels, or 0.39 pound per plant, while 3 plants per hill (10,668 per acre) yielded 52.9 bushels, or 0.28 pound per plant. In other words, under the conditions of his experiments, a uniform two-thirds reduction in stand resulted in an increase in yield per plant of more than 100 per cent.

Just how much increase in yield per plant may be expected from healthy plants under the conditions of the writers' experiments has not been determined. If, however, the reduction in stand is not excessive and the yield per plant following inoculation is below that of the controls, it seems reasonable to assume, on the basis of the greenhouse experiments, that injury to the root system has caused the reduction in yield per plant of the plants not killed in the seedling stage.

Only a very general comparison of the results of field inoculations for the three years can be made, inasmuch as weather conditions for the three years were so different. The mean air temperature for the 10 days following planting in 1924 was approximately 14° C. For the corresponding period following the May 12 planting in 1925 it was 16° rising to 25° for the 10 days after the late planting on June 4. In 1926 the mean temperature for the corresponding period following planting was 19°. The total rainfall for the months of May and June was 10.75 inches in 1924, 2.9 inches in 1925, and 7.1 inches in 1926. Inoculations made before or near the middle of May resulted in considerable reduction in stand, the percentage varying with different strains of corn. Later plantings caused less reduction in stand. Reduction in yield per plant occurred in varying amounts in both early and late plantings, some strains of corn apparently being more susceptible than others.

From the limited field data obtained from parallel inoculations with *Gibberella saubinetii* and *Pythium arrhenomanes* during two seasons, it appears that the percentage reduction in germination is high in the early plantings and that *G. saubinetii* usually reduces the stand to a greater extent than *P. arrhenomanes*. On the other hand, *P. arrhenomanes* may cause a greater reduction in yield per plant in both early and late plantings. *G. saubinetii* attacks the coleorhiza and mesocotyl of the seedling, retarding the development of an extensive root system. *P. arrhenomanes* seldom attacks the mesocotyl but instead rots the smaller feeding roots.

A comparison of the effect of the two fungi on the yield per plant can be made in the few cases in which reduction in stand was practically the same following inoculation with *G. saubinetii* and *P. arrhenomanes*, as shown in Table 8.

TABLE 8.—*Results of inoculating three inbred strains of corn with Gibberella saubinetii and Pythium arrhenomanes, planted May 16, 1924*

Strain	Inoculum used	Field stand		Mean yield per plant (pounds)	Odds [a]
		Number of plants	Mean (per cent)		
B-1-1-3-R-7-3	None	50	60.0	0.563	
	G. saubinetii	18	24.0	.736	>9999:1
	P. arrhenomanes	18	24.0	.514	1666:1
G-8-8-1	None	55	72.0	.502	
	G. saubinetii	42	56.0	.429	5309:1
	P. arrhenomanes	41	54.7	.384	>9999:1
A-1-1-2-R-3-2	None	46	60.9	.513	
	G. saubinetii	32	42.7	.595	3:1
	P. arrhenomanes	31	41.3	.590	26:1

[a] For difference in yield between uninoculated and inoculated plants.

Although both *Pythium arrhenomanes* and *Gibberella saubinetii* (*2*) cause the greatest seedling injury at low temperatures, resistance or susceptibility to these two organisms does not seem to be combined necessarily to the same degree in a given strain of corn.

SUMMARY

Pythium injury to corn may be manifest as (1) a rot of the embryo, preventing germination; (2) as a seedling blight after emergence; or (3) as a root rot that tends to reduce the size, vigor, and yield of the maturing plant.

The causal organism has just been described by Drechsler as *Pythium arrhenomanes* n. sp.

Unlike *Diplodia zeae* and *Gibberella saubinetii*, *Pythium arrhenomanes* attacks the mesocotyl of the seedling only in the late stages of the disease, and then only in cases of severe attack. Infection takes place at the tip of the rootlet. It produces a soft rot that involves first the cortex and later the vascular elements.

Growth of the fungus on potato-dextrose agar takes place between 4° and 40° C. The optimum for vegetative growth lies between 30° and 36°.

Experiments in which open-pollinated and inbred corn was inoculated with *Pythium arrhenomanes*, at the time of planting, in soil-temperature control tanks indicate that soil temperatures near 16° C. or lower, together with high soil moistures, are so favorable for infection that germination of corn kernels may be prevented or seedling blight produced. When inoculated corn is not killed in the seedling stage, the height and dry weight of tops of the plants are reduced. The highest percentage reduction occurred at the lower temperatures. Evidences of differing degrees of resistance or susceptibility were manifest in different inbred strains of corn.

Field experiments during three seasons tend to confirm the results obtained in the greenhouse.

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VARIATION IN SEED FUZZINESS ON INDIVIDUAL PLANTS OF PIMA COTTON[1]

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INTRODUCTION

It was shown in an earlier paper [2] that plants of the Pima variety of American Egyptian cotton, grown under irrigation in Arizona, produced longer lint in the bolls borne on the upper part of the plant than in the lower bolls. The data from which this conclusion was drawn were obtained as follows: The fruiting branches on each of 10 plants were numbered consecutively from the lowest to the highest, the boll borne at the second node of each alternate fruiting branch was harvested when mature, and the lint from each boll was measured. The 10 plants were taken as one array, since they did not differ significantly in their mean lint lengths. The coefficient of correlation (not given in the paper cited) between number (height) of the fruiting branch and lint length for this population was 0.491 ± 0.047, indicating a rather marked tendency for the lint to increase in length from the base to the summit of the plant.[3]

Another character showing considerable variation on the individual cotton plant is the fuzziness of the seeds. A strong tendency to greater fuzziness of the seeds produced in the lower bolls has frequently been noted in Pima cotton grown in Arizona. In an endeavor to obtain statistical evidence of the magnitude and significance of this difference the procedure followed was similar to that used in studying the variation in length of lint.

MATERIAL AND METHODS

Ten plants of an inbred family of the Pima variety (family 5-11) were taken at random at the United States Field Station, Sacaton, Ariz., in 1927. The only conscious selection was the rejection of plants on which very heavy shedding had taken place. On December 10, about 10 days after the first killing frost, every open boll on each of these plants was picked separately, and its position on the plant was recorded on a label attached to the sample. The position was fixed by numbering the fruiting branches consecutively from base to

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[1] Received for publication July 13, 1928; issued November, 1928.

[2] KEARNEY, T. H., and HARRISON, G. J. LENGTH OF COTTON FIBER FROM BOLLS AT DIFFERENT HEIGHTS ON THE PLANT. Jour. Agr. Research 28: 563-565, illus. 1924.

[3] It was noted, however, that the bolls borne on the very highest fruiting branches, which opened late in the season, contained somewhat shorter lint than the bolls borne on most of the upper fruiting branches. This is clearly brought out in the following: KEARNEY, T. H., and HARRISON, G. J. Op. cit., p. 564, fig. 1, Table 2. In this connection, E. C. Ewing of the Delta and Pine Land Company of Mississippi has informed the writers that in the Delta region of Mississippi the lint of long-staple upland varieties of cotton is shorter in the later than in the earlier pickings. The difference in behavior may be due to the fact that in Arizona cotton is grown under irrigation and is not normally subject to drought late in the season, which Mr. Ewing states is of frequent occurrence in Mississippi.

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apex of the plant and by numbering the nodes of the fruiting branches consecutively from the base of the branch outward. The seed cotton from each boll was ginned separately. After ginning, the packets containing the seeds from each boll were placed on a table with the labels face downward, so that the grading in respect to fuzziness could be done without prejudice.

The grading was done by comparing the seeds with a series of standard samples representing the range of grades (1 to 9) that has been observed in the Pima variety. When there was any doubt as to the grade to which a sample belonged, it was always assigned to the higher grade. The grades represented in this material were 3 to 9, and these extremes are shown in Figure 1, which is reproduced from a photograph of the standards themselves.

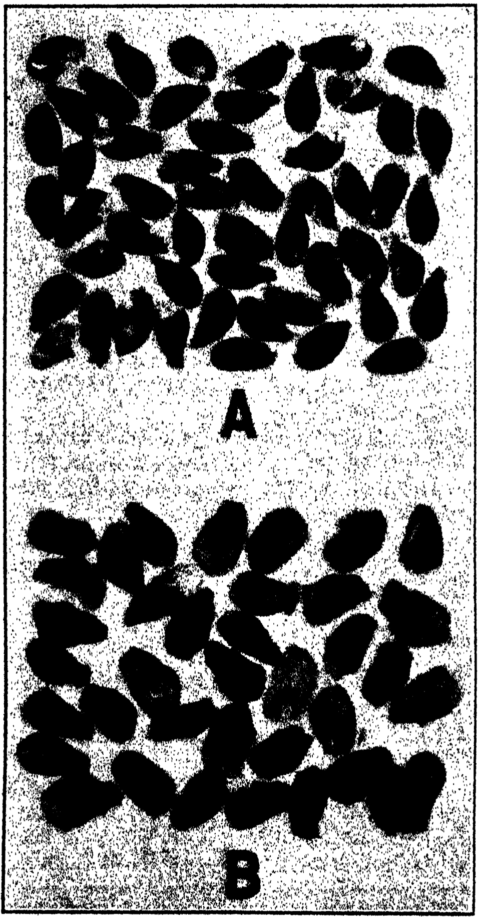

FIG. 1.—Seed fuzziness standards for Pima cotton, showing grades 3 (A) and 9 (B), these being extremes represented on the plants that afforded the data considered in this paper

On January 5, 1928, the bolls were collected from 10 plants of another but closely related Pima family (family 5–12), and this additional material was treated similarly in all respects.

The considerable individual variation in seed fuzziness which characterizes this variety of cotton, manifesting itself even on the plants of inbred and otherwise uniform families, is shown by the fact that on 13 of the 20 plants all seven grades (3 to 9) were represented, while the remaining plants each showed a range of six grades.

STATISTICAL CONSTANTS FOR THE TWO FAMILIES

The mean and standard deviation for each plant and for all 10 plants of each family as one array are given in Table 1. In neither group was the population perfectly homogeneous. The means of two individuals of family 5–11 differed significantly from the mean of the population, the mean of plant 8 having been lower and that of plant 10 higher. In both cases the departure was about five times its probable error. In family 5–12, plant 11 had a significantly higher mean and plants 18 and 19 had significantly lower means than the mean of the population, the departure having been respectively 6.3, 5.3, and 5.3 times its probable error. Nevertheless, for the reason to be given presently, it was decided to treat the 10 plants of each family as one array in determining the correlations.

Comparison of the means of the two populations, as given at the bottom of Table 1, shows that family 5–12 had slightly fuzzier seeds than family 5–11, and that the difference in mean grade was significant, having amounted to 8.6 times its probable error.

TABLE 1.—*Statistical constants for grade of seed fuzziness of 20 plants of Pima cotton*

Family and plant	Number of bolls	Mean grade	Standard deviation [a]	Family and plant	Number of bolls	Mean grade	Standard deviation [a]
Family 5–11:				Family 5–12:			
No. 1	41	6.0±0.19	1.80	No. 11	76	7.1±0.10	1.34
No. 2	51	5.7± .14	1.47	No. 12	69	6.5± .14	1.71
No. 3	52	5.8± .14	1.54	No. 13	67	6.7± .13	1.57
No. 4	62	5.8± .12	1.37	No. 14	68	6.0± .14	1.78
No. 5	54	6.4± .17	1.89	No. 15	72	6.5± .12	1.48
No. 6	47	5.8± .15	1.49	No. 16	57	6.6± .14	1.59
No. 7	46	5.9± .19	1.88	No. 17	67	6.6± .14	1.74
No. 8	37	4.8± .20	1.83	No. 18	63	5.6± .15	1.82
No. 9	46	5.6± .16	1.61	No. 19	60	5.6±9.15	1.70
No. 10	51	6.6± .14	1.54	No. 20	80	6.7± .13	1.70
As one array	487	5.87± .05	1.67	As one array	679	6.42±.04	1.70

[a] The standard deviations and probable errors of the individual plant means have been increased by using Pearson's correction for the standard deviation when the numbers are small. See PEARSON, K., ON THE DISTRIBUTION OF THE STANDARD DEVIATIONS OF SMALL SAMPLES: APPENDIX I TO PAPERS BY "STUDENT" AND R. A. FISHER. Biometrika 10: 529. 1915.

CORRELATION BETWEEN SEED FUZZINESS AND POSITION OF THE FRUITING BRANCH

For the 10 plants of each family as one array, the correlations were determined between the height of the fruiting branch as indicated by its number from the base and the grade of fuzziness of the seeds from the bolls borne on the branch in question. The correlations with the height of the branch were determined for (A) the average of the grades of all bolls on the branch and (B) the grade of the boll at the first node of the branch. Since in many cases node 1 was vacant, because of shedding, the numbers are smaller for correlations B than for correlations A. The coefficients obtained are given in Table 2. They are in all cases negative and highly significant, indicating a strong tendency for the fuzziness of the seeds to diminish from base to apex of the plant.

TABLE 2.—*Coefficients of correlation of the grade of seed fuzziness with the position (number from the base of the plant) of the fruiting branch on 20 plants of Pima cotton in 1927*

Height of branch correlated with seed fuzziness as expressed by—	Family 5–11 (plants 1 to 10)			Family 5–12 (plants 11 to 20)		
	n	*r*	*r*/E	*n*	*r*	*r*/E
Average for all bolls on the branch (A)	194	−0.652±0.028	23	216	−0.638±0.027	23.5
Boll at node 1 only (B)	186	− .557± .034	16	164	− .711± .037	19

The observed and fitted regressions of seed fuzziness on the height of the fruiting branch in the two Pima families are shown in Figure 2. The fitted regressions indicate an average decrease in seed fuzziness for each successive pair of branches up the plant amounting to 0.28 grade in family 5-11 and 0.22 grade in family 5-12.

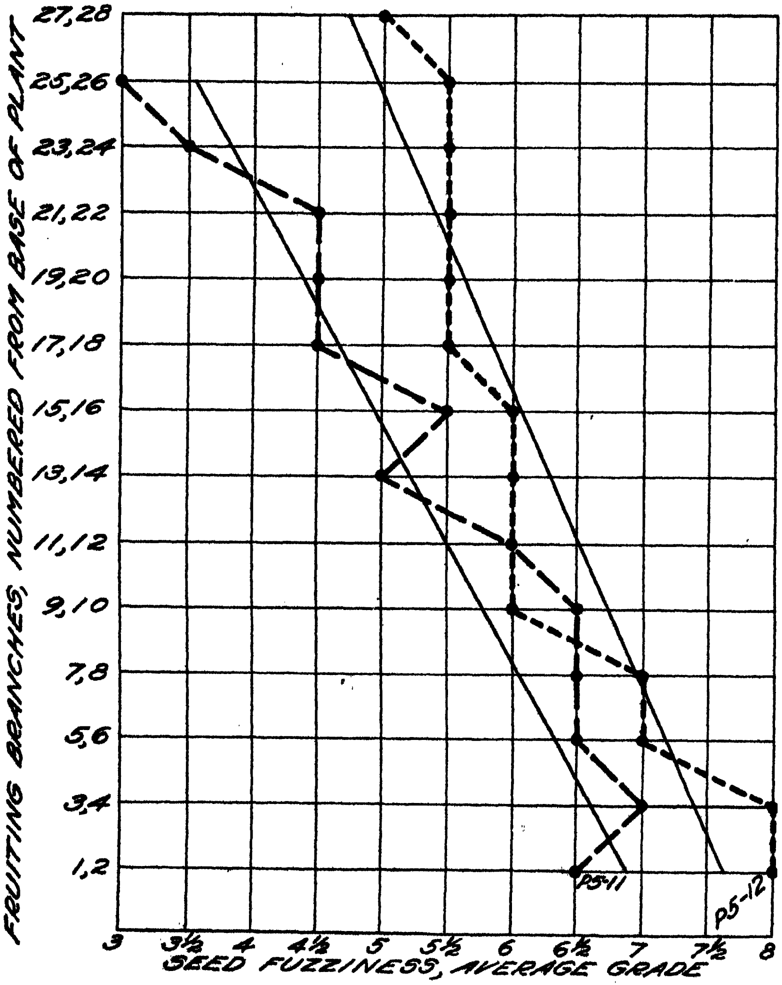

FIG. 2.—Observed and fitted regressions of seed fuzziness on the height of the fruiting branch in Pima cotton. The fitted regressions indicate the average decrease in fuzziness on successively higher fruiting branches. The broken line represents the observed regression for the 10 plants of family 5-11 and the dotted line the observed regression for the 10 plants of family 5-12. The solid lines represent the fitted regressions

It was mentioned in a preceding paragraph that certain plants in each population were somewhat aberrant in their means for seed fuzziness. The correlations between the height of the fruiting branch and seed fuzziness (average of the grades of all bolls on the branch) were determined separately for these individuals and are shown in comparison with the corresponding coefficients of the respective populations (10 plants as one array) in Table 3. The individual coefficients in no case differ significantly from the coefficient of the whole population and, like the latter, are in every case negative and significant. Therefore, it seemed justifiable to include these plants in the populations on which were determined the correlations given in Table 2.

TABLE 3.—*Coefficients of correlation between the height (number) of the fruiting branch and seed fuzziness for the apparently aberrant plants in Pima families 5–11 and 5–12 and for the 10 plants of each family as one array*

Family and plant	Coefficient	Family and plant	Coefficient
Family 5-11:		Family 5-12:	
Plant 8	−0.699±0.084	Plant 11	−0.597±0.091
Plant 10	− .520± .110	Plant 18	− .629± .093
		Plant 19	− .791± .055
Ten plants as one array	− .652± .028	Ten plants as one array	− .638± .027

CORRELATION BETWEEN SEED FUZZINESS AND POSITION OF BOLL ON FRUITING BRANCH

In order to ascertain whether there is a tendency for the seed fuzziness to vary consistently at different points on the same fruiting branch, the following procedure was adopted: The nodes of the individual fruiting branches were numbered consecutively outward, and the correlation between the node number of the boll and the grade of fuzziness of the seeds contained in it was determined without regard to the position of the branches themselves. The correlation for 487 fruiting-branch nodes in family 5–11 (plants 1 to 10) gave a coefficient of −0.290±0.028 (*r*/E 10.3); and the correlation for 679 fruiting-branch nodes in family 5–12 (plants 11 to 20) gave a coefficient of −0.420±0.021 (*r*/E 20.0). There is, therefore, a tendency for the bolls at nodes near the base of the branch to contain fuzzier seeds than the bolls farther out on the branch.

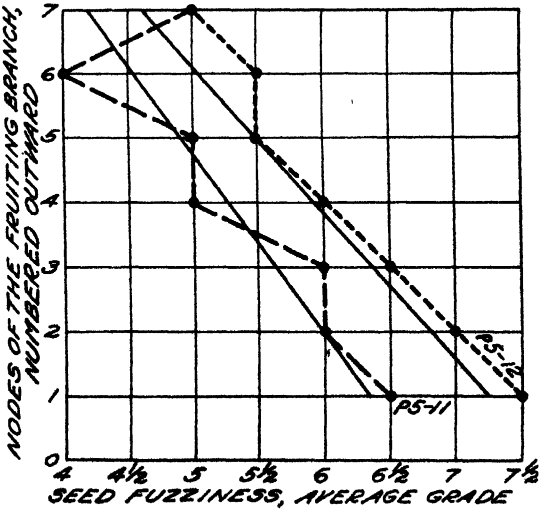

FIG. 3.—Observed and fitted regressions of seed fuzziness on the node of the fruiting branch in Pima cotton. The fitted regressions indicate the average decrease in fuzziness at successsive nodes outward on the fruiting branch. The broken line represents the observed regression for the 10 plants of family 5–11 and the dotted line the observed regression for the 10 plants of family 5–12. The solid lines represent the fitted regressions

The observed and fitted regressions of seed fuzziness on the number of the node of the fruiting branch in the two Pima families are shown in Figure 3. The fitted regressions indicate an average decrease in seed fuzziness for each successive node outward on the branch amounting to 0.36 grade in family 5-11 and 0.43 grade in family 5-12.

Additional and conclusive evidence of this relation was obtained by comparing, for all branches retaining three or more bolls, the seed fuzziness grade of the innermost and the outermost boll. The numbers of such branches available were 78 on the 10 plants of family 5-11 and 113 on the 10 plants of family 5-12. The means of the grades of the innermost and outermost bolls are stated in Table 4, the probable errors of the differences having been computed from the array of differences between pairs (innermost and outermost boll of each branch). It is evident that the inner bolls averaged about two grades fuzzier than the outer bolls and that the differences are highly significant.

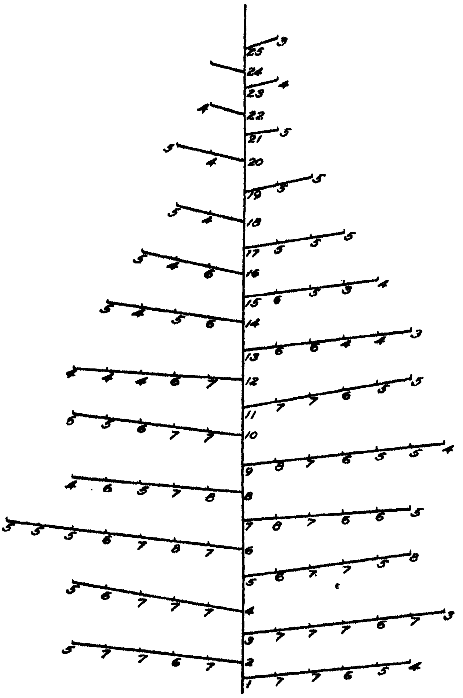

FIG. 4.—Composite diagram of 10 plants of Pima cotton (family 5-11), showing the average grade of seed fuzziness of the boll at each node of each fruiting branch. The vertical line represents the main stalk, and the numbers along it are those of the successive fruiting branches. The lines to the right and the left represent the fruiting branches, and the figures along them show the average of the grades of seed fuzziness (nearest whole number) for all bolls in the population which were borne at the position indicated

The relation between the position of the boll and the fuzziness of the seeds is shown in Figure 4, which is a composite diagram of the 10 plants of family 5-11. The vertical line represents the main stalk and the figures along it are the numbers of the fruiting branches. The lines to the right and the left represent the branches, and the figures along them show the average of the grades of seed fuzziness (nearest whole number) of all bolls in the population borne at the position indicated. There having been considerable shedding of buds and bolls on all of these plants, the averages in several cases are based on only one or two bolls. This doubtless accounts for most of the irregularity of the gradation in seed fuzziness upward on the plant and outward on the branches.

TABLE 4.—*Mean grade of fuzziness of seeds from bolls nearest and farthest from the base of each fruiting branch bearing three or more bolls*

Position of bolls	Mean grade of seed fuzziness in—	
	Family 5-11 (plants 1 to 10)	Family 5-12 (plants 11 to 20)
Nearest base of branch	7.21	7.66
Farthest out on branch	5.04	5.76
Difference	2.17±0.15	1.90±0.12
D/E	14.5	16

SUMMARY AND CONCLUSIONS

Evidence is presented in this paper which shows that in Pima cotton grown in Arizona there is pronounced variation, as between bolls borne in different positions on an individual plant, in the quantity of fuzz or short hairs on the seeds. Rather high and very significant negative correlations between the height of the fruiting branch and the grade of fuzziness of the seeds borne thereon indicate a strong tendency for the bolls on the lower fruiting branches to have fuzzier seeds than the bolls on the higher branches. Lower but still rather significant negative correlations between the number of the node on the individual fruiting branch and the fuzziness of the seeds contained in the boll at that node show that seeds produced nearest the base of the branch tend to be fuzzier than those produced farther out on the branch.

These positional relations suggest that better conditions of nutrition may be conducive to greater development of fuzz on the seeds, since it is the bolls borne on the lower part of the plant and near the base of the fruiting branch that usually have the fuzziest seeds. Conversely, the quantity of fuzz tends to be smallest on seeds produced farthest from the roots and from the main stalk of the plant. It is not improbable, however, that changes in temperature or in the length of day as the season advances may be important factors, since the bolls produced near the base of the plant and of the fruiting branch develop while the day and night temperatures are highest and the days are longest; whereas temperatures, particularly night temperatures, are appreciably lower and the days are appreciably shorter during the period of development of the bolls near the top of the plant and farthest out on the fruiting branches.

The flowers that open late in the season, hence on the highest fruiting branches and at the farthest nodes of lower branches, are smaller and paler colored than the earlier flowers. So far as the writers know, no comparison has yet been made of the size and weight of the bolls and weight of the seeds in relation to position on the plant. Data are at hand, however, in regard to the time required for maturation of the boll. Martin, Ballard, and Simpson [4] found that in several types of cotton the maturation period (number of days from opening of the flower to opening of the boll) increases as the season advances. It is stated that in Pima cotton grown at Phoenix, Ariz., C. J. King observed the maturation period to increase from 54 days for flowers opening in July to 82 days for flowers opening in September. Observations on Pima cotton by R. H. Peebles and Max Willett at the United States Field Station, Sacaton, Ariz., in 1921, showed the maturation period to lengthen from an average of 54.5 days for 618 flowers opening in July to an average of 74.5 days for 981 flowers opening in September.

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[4] MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M. GROWTH OF FRUITING PARTS IN COTTON PLANTS. Jour. Agr. Research 25: 203-206. 1923.

Most of the flowers opening in July are borne on the lower fruiting branches and on the basal nodes of the branches, whereas most of the flowers opening in September are borne on fruiting branches near the top of the plant or far out on lower branches. Consequently, as in the case of seed fuzziness, it would be possible only by experimental control of temperature and of the daily period of illumination to ascertain the relative importance of these factors and of conditions with respect to nutrition in determining the length of the maturation period.

If relatively unfavorable nutritional or meteorological conditions are responsible for the slower maturation of the bolls from flowers produced late in the season and high on the plant and for the smaller quantity of fuzz on the seeds contained in these bolls, it is difficult to account for the fact, mentioned in the introduction to this paper, that on plants of Pima cotton grown in Arizona the lint contained in the upper bolls was found to be longer than that in the lower bolls. A negative correlation between lint length and seed fuzziness would be expected if the increase in length of lint upward on the plant is a general phenomenon, but in the two instances in which the correlation between this pair of characters has been determined for Pima cotton in Arizona the coefficients obtained were positive, although not significant (r 0.046 ± 0.051 and r 0.073 ± 0.047). It would be unprofitable to speculate further concerning this apparent discrepancy until determinations of lint length and seed fuzziness have been made on the same individual bolls borne in different positions on the plant.

The magnitude of the variation in seed fuzziness from boll to boll on the same individual is noteworthy. Of the 20 plants examined in this investigation, 13 showed a range of seven grades and 7 showed a range of six grades, the total variation which has been observed in the Pima variety being represented by nine grades. It is evident that in comparing different strains or varieties of cotton in respect to this character, and in genetic studies, particular care must be used in collecting the seeds. These should be taken either from bolls occupying corresponding positions on the several individuals or from a sufficient number of bolls on all parts of the plant to afford an average sample.

ZONATE EYESPOT OF GRASSES CAUSED BY HELMINTHOSPORIUM GIGANTEUM[1]

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INTRODUCTION

Helminthosporium giganteum Heald and Wolf (*6*)[2] was described in 1911 from Texas, where it was found occurring on diseased Bermuda grass (*Cynodon dactylon* L.), as the cause of lesions evidently of the eyespot type. The writer included a discussion of the fungus in a comparative account published in 1923 (*4*), in which its occurrence on goose grass (*Eleusine indica* (L.) Gaertn.) and quack grass (*Agropyron repens* (L.) Beauv.) was noted and the peculiar mode of germination characteristic of its conidia was described. In an abstract that appeared somewhat earlier (*3*) the parasite had been reported on nearly a score of additional species of grasses and an explanation offered of its method of extension as prevailing in the development of a much more destructive type of injury observed on several hosts and designated as zonate eyespot. In the present paper the degree of injury sustained by the grasses on which the fungus has hitherto been observed to occur naturally will be more fully discussed, together with certain features pertaining to the morphology and develoment of the parasite.

DISTRIBUTION AND SEASONAL OCCURRENCE OF PARASITE

Such fragmentary information concerning the distribution of *Helminthosporium giganteum* as it has been possible to obtain in occasional field trips undertaken for other purposes indicates that in the United States it is largely restricted to the southern and middle latitudes. Collections made by the writer at Seaford, Del., in August, 1922; at Hurlock, Md., in August, 1923; in the District of Columbia and neighboring sections of Virginia and Maryland in 1922, 1923, 1924, 1925, and 1926; and at Menfro, Mo., in August, 1924, provide clear evidence that the parasite is not limited to a strictly southern distribution. Precisely how much farther north its natural distribution extends is not known except that efforts to find the fungus in the western portion of Long Island during the seasons of 1920 and 1921, in the vicinity of Vincennes, Ind., in August, 1924, and in the vicinity of Allentown, Pa., in September, 1925, were unsuccessful, although grasses capable of serving as congenial hosts

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[1] Received for publication Apr. 27, 1927; issued November, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 492.

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were present in quantity.[3] On the other hand, except apparently for mountainous and elevated regions, the parasite has been found of widespread occurrence in the Southeastern States from Virginia to Florida, while its prevalence in a number of localities in southeastern Missouri, visited in August, 1923, suggests a parallel distribution in the Mississippi Valley.

Helminthosporium giganteum shows much variability in the abundance of its occurrence from season to season. During the summer of 1922 it appeared in the vicinity of Washington, D. C., as probably the most destructive single fungous parasite affecting the Gramineae as a family, its attack on several of its hosts being of extreme severity, while on some others its attack, though less destructive, was nevertheless severe. During the seasons of 1923, 1924, 1925, and 1926 the fungus was far less prevalent and, in general, of only minor importance, although in a number of situations, as along the banks of the Chesapeake & Ohio Canal, it reappeared from year to year in nearly undiminished quantity. In addition to irregularity with respect to seasonal occurrence, it exhibits much less uniformity in local distribution than the large majority of parasitic fungi. Even in the season of 1922, when in many locations stands of Bermuda grass were all but killed as a result of the ravages of the parasite, it was not an unusual experience to find other stands of the same grass within a distance of less than 100 meters free of injury. Such pronounced inequality of distribution has been found characteristic of the fungus wherever adequate observations have been made, prevailing apparently in southeastern Missouri exactly as in Virginia and Maryland.

For such localized distribution a partial explanation may be offered. Compared to some of the more nearly ubiquitous types of foliar parasites—as, for example, *Helminthosporium sativum* P. K. & B.—*H. giganteum* produces even under favorable conditions a relatively small number of spores. These spores, as has been pointed out previously (*4, p. 676*), are the shortest lived spores of any species of Helminthosporium hitherto encountered by the writer. There is evidence, too, that they are not well adapted for extensive dispersal. The dissemination of conidia of *H. giganteum* can be studied to advantage in situations where a single isolated infected stand of a favorable host on which active sporulation is taking place is found adjacent to, or surrounded by, species of grasses allowing the production of only incipient lesions devoid of fructifications. The abundance or scarcity of sterile lesions on the uncongenial grasses in such circumstances may be regarded as a reliable index of the quantity of spores reaching any particular spot from the stand of the congenial species of grass. The numbers of such lesions fall off rapidly beyond distances of 1 to 2 meters; few are to be observed at a distance of 5 meters; only a vanishing quantity can be found at 10 meters, while none have ever been observed at a distance of 20 meters. It is scarcely to be doubted that the unusual size and consequent relatively great weight of the conidia are in large part responsible for the restriction in spread. Obviously these bodies would scarcely remain suspended long in the somewhat quiet atmosphere often associated with the light, sustained precipitation that provides optimum conditions for their germination.

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[3] As a pest affecting creeping bent grass in putting greens of golf courses, the fungus was found to occur during the season of 1928 in widely separated localities in the northern tier of Middle Western States. In the three localities where the writer had occasion to make observations, viz, La Fayette, Ind., Detroit, Mich., and Wooster, Ohio, natural stands of susceptible hosts (as, for example, quack grass) showed no evidence of infection with zonate eyespot. Nor were any signs of attack by *Helminthosporium giganteum* evident in the creeping bent immediately surrounding affected putting greens but not exposed to artificial watering. It appears highly probable that the success of the fungus well north of what would seem to be its natural range is contingent on the copious irrigation usual in the management of greens. As the grass is generally propagated by stolons, and since these to a large extent have been distributed from sources within the natural range of the parasite, the means by which the introduction of the latter into northern localities has been effected are sufficiently obvious.

OVERWINTERING OF PARASITE

In the vicinity of the District of Columbia *Helminthosporium giganteum* ceases to develop vegetatively or to produce conidia with the advent of cool weather during the early part of October. The fungus appears to overwinter as dormant mycelium. At intervals during the spring of 1923, quack-grass leaves of the previous season, with well-developed lesions, were collected near Cabin John, Md., brought into the laboratory, and incubated in a moist chamber. Fresh conidia were obtained in this way until early in May, when, with the appearance of lesions on the new quack-grass foliage, the trials were discontinued. Although it was never possible to determine definitely that fresh conidia were not proliferated from the old conidiophores, most of the conidia were apparently produced on new conidiophores, and perhaps all may have had such origin.

DEVELOPMENT OF LESIONS IN RELATION TO SPECIFIC SUSCEPTIBILITY

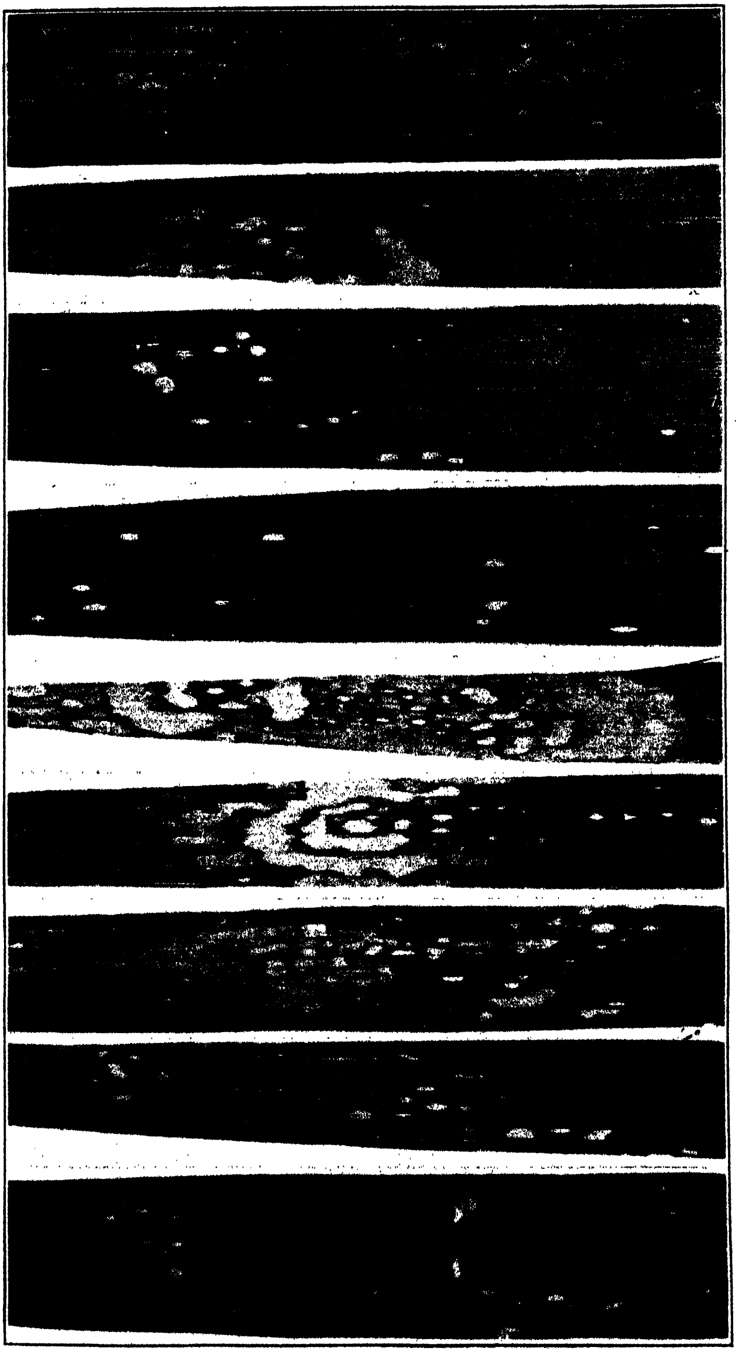
Collectively the various grasses (pls. 1–7) found to show evidence of attack by *Helminthosporium giganteum* under natural conditions manifest the widest range in degree of susceptibility. The most general manifestation of an individual infection is the appearance on the foliage of a minute longitudinally elongated spot, the size and coloration of which vary with the host. In *Muhlenbergia schreberi* Gmel., for example, this spot first becomes visible as a sharply defined very dark speck, often not exceeding 0.05 mm. in width and 0.2 mm. in length. (Pl. 5, N–R.) Through subsequent enlargement it may attain a length of approximately 1.2 mm. and a width of 0.1 to 0.2 mm., then often revealing within these relatively minute dimensions a decolorized central region. In other hosts, where discoloration is less intense, the spots, when first recognizable, may be somewhat larger and less sharply delimited than in *M. schreberi*, and the primary lesions, before attaining definitive size, may become several times larger. The fading of the central region to yield the eyespot type of lesion generally distinctive of the disease occurs with less regularity in some hosts than in others. *Panicum dichotomoflorum* Michx., for example, as a result of frequent omission of this development, often exhibits the spot-blotch type in larger number. In timothy (*Phleum pratense* L.) the lesion is practically devoid of dark discoloration, being present as a dead region from which the normal green coloration has disappeared.

The mere occurrence of the eyespot type of lesion on a grass does not necessarily provide evidence of its suitability to serve as a host to *Helminthosporium giganteum*. In the case of many species of grasses such lesions have never been discovered except in mixed stands with more congenial hosts, on which the parasite is not only present but sporulating abundantly as well. In Virginia, Maryland, and Missouri, Bermuda grass, goose grass, and quack grass appear to serve most frequently as sources of infection, most of the writer's observations on the response of other grasses having consequently been carried out when the latter occurred in immediate proximity to these widely distributed weeds, and most frequently as intimate admixtures. Under such circumstances the foliage of uncongenial hosts may bear an abundance of spots, as occurs, for example, in *Panicum clandestinum* L., or a few more remotely scattered ones may be produced, as in timothy. In any case the tissue involved never appears to give rise to fructifications of the fungus, nor can such structures ordinarily be obtained by incubation in a moist chamber. If the affected leaves are still green, the identification of the parasite may usually be accomplished, though somewhat laboriously, by isolating it from bits of tissue excised from the margins of lesions and planted on a suitable culture medium after surface sterilization followed by thorough washing with sterile water.

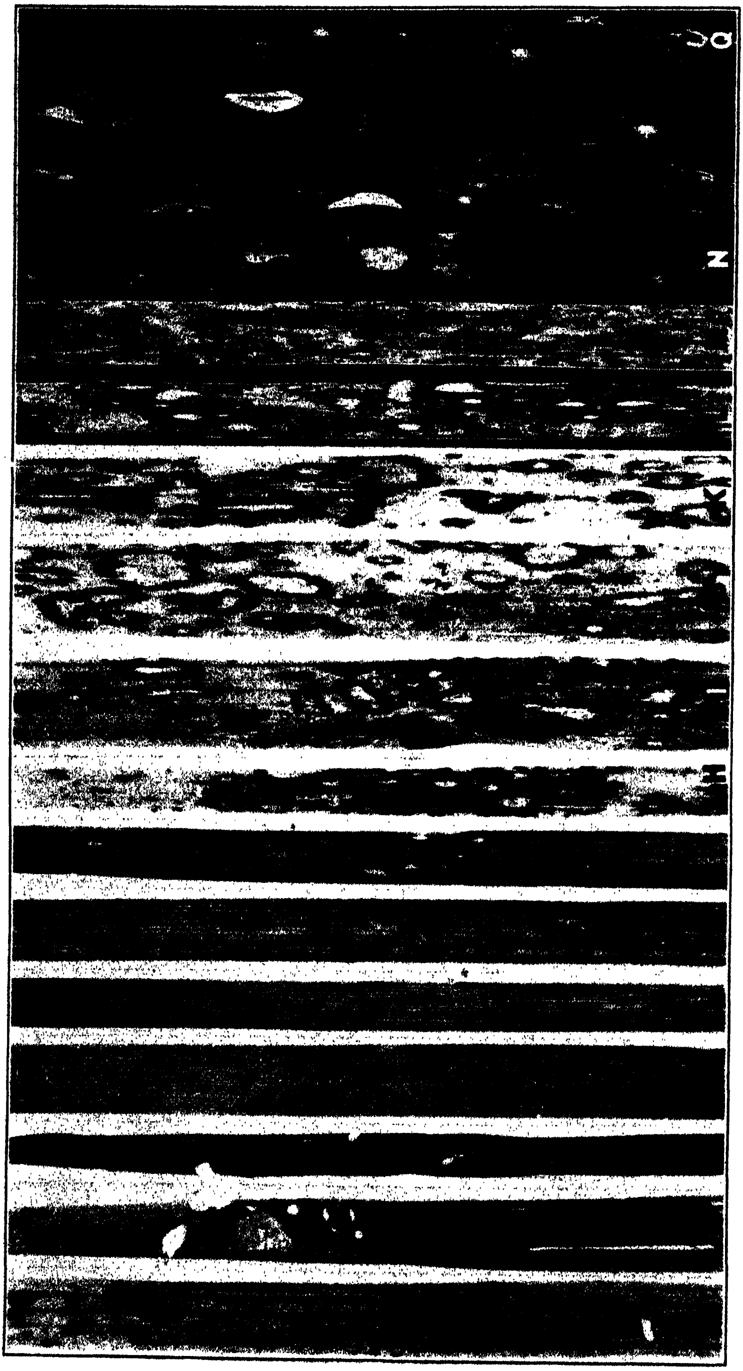
A readier and more certain method of determining the parasite under consideration as the effective causal agent is that of direct microscopic examination; for, owing to the extraordinary size of the conidia, the evacuated collapsed membranes of the individual spore usually can be discerned without the least difficulty on one surface or the other of the lesion that it produced. The examinations, it may be mentioned, revealed that very generally the greater number of infections result from spores on the upper or adaxial surface of the leaf, a fact to be attributed, perhaps, to the somewhat more effective exposure of this surface to air-borne bodies. On whatever surface the spore membranes may be found, however, they are always securely fastened to the host by the evacuated germ tubes, so that alcohol and clearing agents may be employed to improve the optical features of the material without incurring any risk of washing off the structures in question.

Most frequently the evacuated spore membranes occupy an approximately median position relative to the foliar lesion, the germ tubes from both ends having evidently been equally active in killing regions of tissue that became sufficiently extensive to coalesce into one. However, in the case of hosts in which, as in *Muhlenbergia schreberi*, the lesions are clearly delimited and very narrow, such coalescence does not always take place, particularly when the conidium is oriented in a direction transverse to that of the leaf. In such cases two distinct lesions separated by a distance not usually exceeding 0.3 mm., yet clearly evident as two to the naked eye, result from the germination of the single conidium. This condition is infrequent in fungous diseases generally, and manifestly is not readily possible with fungi having spores of more ordinary dimensions. A similar pairing is exhibited also by newly developed lesions on more favorable hosts (pl. 1, A), but owing to the enlargement and fusion of the individual spots the binary arrangement later becomes obliterated. Sometimes a spore may be more or less eccentric in position with reference to the lesion produced by it, evidently as a result of irregularities in germination due to accidents attending the process or to the previous death of some of the segments.

Among the uncongenial hosts considerable difference exists with respect to the number of lesions produced under circumstances equally favorable for infection. *Panicum dichotomoflorum* and *Muhlenbergia*



Portions of leaves of *Phalaris arundinacea* attacked by *Helminthosporium giganteum*. A–E, Series of specimens showing infection of increasing severity; F–H, Water-soaked zones surrounding lesions as a result of incubation in moist chamber for 16 hours; I, Leaf completely involved in extensive lesions with conspicuous zonate markings; × 2



Leaves of various grasses attacked by *Helminthosporium giganteum.* A–C, *Agropyron elongatum,* × 2; D–H, *A. intermedium,* × 2; I–M, *A. repens,* × 2; N–Q, *Agrostis stolonifera,* × 4 *schreberi*, when found in mixed stands with heavily infected Bermuda grass, are generally very liberally peppered with discolored spots, the lesions here being often as abundant as on the congenial hosts. On the other hand, *Panicum gattingeri* Nash, as well as timothy and Kentucky bluegrass (*Poa pratensis* L.), under the same conditions exhibit only a meager sprinkling of spots. Neither crabgrass (*Digitaria sanguinalis* (L.) Scop.) nor *Chaetochloa lutescens* (Weigel) Stuntz were ever found spotted by the fungus in the vicinity of Washington, D. C., during the five successive seasons in which observations were made. Near Kennett, Mo., however, lesions attributable to conidia of the parasite were found on both, though, to be sure, in small number. It is probable that such facts of presence or absence on a particular host may involve only casual details of distribution. On the other hand, they may point toward differences in environmental conditions, or toward possible differences in the biological constitution either of the parasite or of the grass host. In the absence of more precise information, it may be mentioned in this connection that, on the whole, the distribution of the fungus in nature does not suggest the existence of physiological varieties or races paralleling generic divisions in the Gramineae.

In the case of the more congenial hosts, the early stage in the establishment of the parasite is closely similar to the development of the small lesions just described. (Pl. 1, A, B.) The hyphae proceeding from the germinating conidium here also bring about the discoloration and death of a limited tract of tissue. On isolated eyespot lesions of such origin, fructifications of the fungus do not ordinarily arise. However, when, as in Bermuda grass, these spots become numerous and crowded, causing the leaf involved to wither somewhat generally, conidiophores appear in considerable abundance, from intervening regions as well as from the bleached areas included within the lesions.

A generally more copious production of sporophores and spores takes place on leaf tissue directly killed by the parasite as a result of a peculiar type of secondary development. This type of development is most strikingly exemplified on *Phalaris arundinacea* L. and appears to be dependent on the presence of liquid water on the surface of the leaf. When, because of heavy dews or prolonged drizzling rains, a layer of water persists 12 hours or more on infected foliage of reed canary grass, many of the eyespot lesions will be found surrounded by an enlarging water-soaked zone. (Pl. 1, F, G, H.) Microscopic examination of the surface of the leaf reveals the presence of hyphae arising near the edge of the original lesion and traversing the water-soaked zone radially to its margin, giving off branches in their course. These superficial filaments adhere very closely to the epidermis of the host, and would seem to communicate with the interior of the leaf by branches penetrating the epidermis, although the direct optical evidence for such communication is far from satisfactory. In any case, the water-soaked zone is rather accurately coextensive with the region included in the centrifugal growth of superficial hyphae. With the disappearance of the layer or film of water on the advent of drier conditions, growth of the superficial mycelium ceases and the zone of water-soaked tissue dries up, thus becoming the peripheral belt of the enlarged lesion. (Pl. 1, D.) When, as in the season of 1922, weather conditions are such as to permit repeated occurrence of the same cycle of development, many of the leaves become entirely involved, the dead foliage, entirely covered with intricately zonate patterns, presenting a most distinctive aspect. (Pl. 1, E, I.) Irregularities in such patterns (pl. 1, D) are attributable, as might be expected, to the casual distribution of the moisture deposited, regions failing to become covered being recorded as interruptions in the zone developed during any particular moist period under consideration. The older leaves of reed canary grass usually show most extensive infection on the blade midway between base and tip. Field inspection has shown that this median portion also becomes more liberally bathed in dew, a fact due apparently to the drooping habit of the distal part and the accumulation of moisture near the keystone position on the resultant arch.

The importance of such secondary development in the biology of the parasite is considerable, as by far the larger portion of the conidiophores and conidia are produced in the extensive regions of host tissue killed thereby. The degree to which secondary development takes place on any host becomes thus a truer measure of its congeniality than the number of infections. Hosts that may well be regarded as congenial include, in addition to Bermuda grass and reed canary grass, *Agropyron repens*, *A. intermedium* Beauv., *A. elongatum* Host, *Bromus inermis* Leyss., *Eleusine indica*, *Echinochloa crusgalli* (L.) Beauv., *Elymus virginicus* L., *Lasiagrostis splendens* Kunth,[4] and *Leersia virginica* Willd. Under favorable conditions all of the grasses mentioned would seem to permit the parasite to propagate itself indefinitely. Sporulation was observed also on leaves of *Eragrostis major* Host and of *Muhlenbergia mexicana* (L.) Trin., though in such meager quantity as to render doubtful the capacity of the parasite to maintain itself on these grasses, except possibly under most favorable conditions.

ISOLATION AND ARTIFICIAL CULTIVATION OF PARASITE

The isolation of the parasite, though not excessively difficult, usually can not be accomplished with as much ease as the isolation of graminicolous species of Helminthosporium generally. Plantings made on a suitable agar medium, like maize-meal agar, with small pieces of tissue dissected from the margins of growing lesions, after surface sterilization and washings in repeated changes of sterile water, while not uniformly successful, afforded the most convenient means of securing pure cultures. The fungus, on growing out of the tissue, is recognizable by the fringe of aerial mycelium, composed of filaments of relatively large, unvarying diameter, with a distinctive branching habit, and disposed in snarls of numerous and often graceful curves. The mycelium immersed in the substratum lacks this disposition, but shows a similar degree of uniformity in diameter and a similar type of branching, with the contents generally homogeneous and moderately refringent. (Fig. 1.) Transferred to fresh media, the mycelium retains these tendencies. As growth is relatively slow, even at optimum temperatures, which seem to lie between 25° and 29° C., the snarled aerial mycelium, except at the growing margin, is usually dried out and collapsed. It then appears to the naked eye as a somewhat granular or flaky white or grayish material, sprinkled

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[4] The binomial under which the plantings of this grass at the Arlington Experiment Farm were recorded and under which it was reported (*3*) as a host of *Helminthosporium giganteum* is retained here. Specimens kindly examined by A. S. Hitchcock were referred by him to *Stipa splendens* Trin.

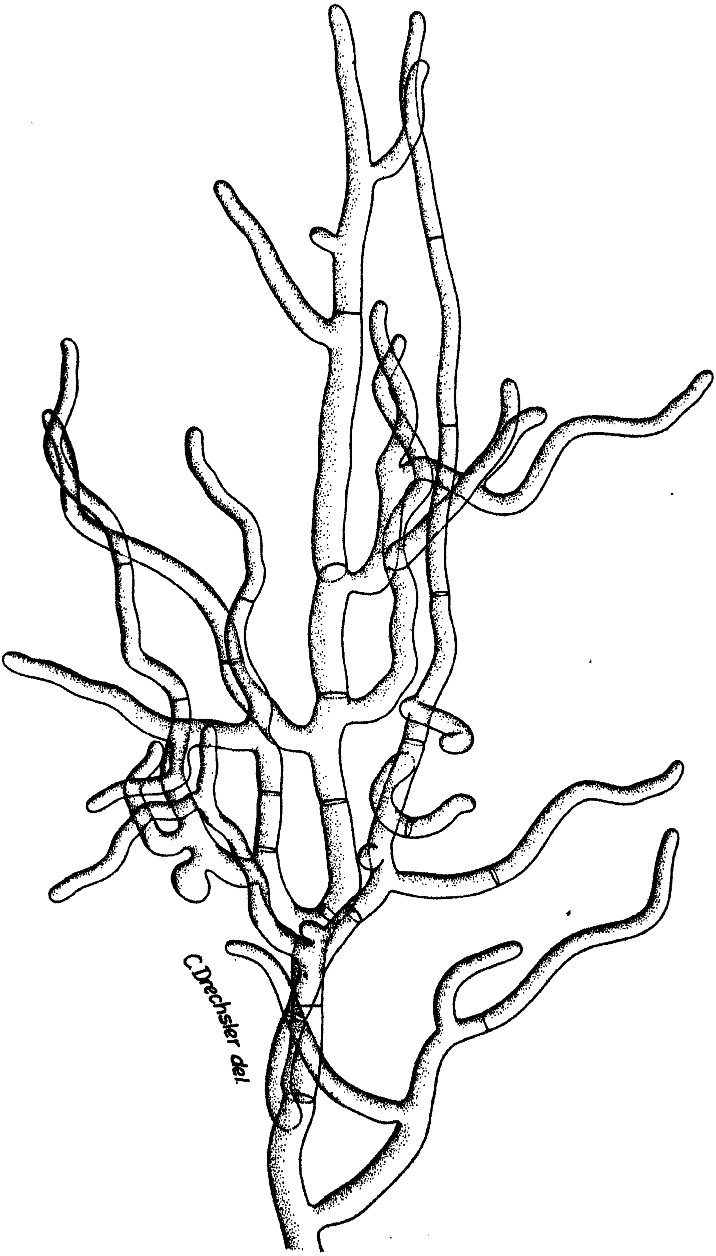

FIG. 1.—Portion of the submerged mycelium of *Helminthosporium giganteum* from the margin of growth on maize-meal agar. × 450

irregularly over the surface of the culture. (Pl. 8.) The submerged mycelium in the older portions of the culture is somewhat dark, and under the microscope appears well provided with septa, though apparently largely devoid of protoplasmic contents. A certain degree of zonation, involving both submerged and aerial mycelium, usually is evident.

Sporulation of *Helminthosporium giganteum* in culture generally is rather meager, but presents interesting features. The sporophores found scattered here and there consist of prolongations or branches of ordinary hyphae (fig. 2, D) from which they usually differ only slightly in a darker coloration and a somewhat thicker membrane. Many of the conidia are not markedly different from conidia developed under natural conditions. (Fig. 2, A–C.) Others, however, are markedly inferior in length and width. An irregular type of proliferation, evidently akin to germination, is frequent. I many instances this is expressed in the production, from the basal and apical segments where the whorl of germ tubes ordinarily arises, of two, three, or four structures that from their suggestive resemblance to conidia might be regarded as secondary conidia. (Fig. 2, C.) These may in turn become proliferous. The repetition of this process, accompanied by marked diminution in size, frequently gives rise to a ramifying system, of which the terminal elements, sometimes as little as 3.5 μ in length and 2.5 μ in diameter, are borne in short branching chains. (Fig. 3, B.) The apparatus thus produced shows marked similarities to fructifications of Hormodendron, not only in the origin of new elements by lateral and apical budding, but also in the ready disintegration of the parts. Branching systems of the same type, but without any of the larger intermediate elements resembling the conidia typical of the fungus, also are produced in some quantity directly from conidia (fig. 3, C, D) or on relatively undifferentiated mycelial branches (fig. 2, E, F; fig 3, A).

As to the possible bearing of the Hormodendronlike structures on the biology of the parasite, no information is available. So far no tendency toward proliferation other than normal germination has been observed in material collected in the field. Most of the writer's observations, however, have been made near the northern limit of the fungus, and it is not impossible that in regions of higher temperature and greater humidity the proliferous tendency may be more pronounced. In any case, regardless of its interest as a morphological detail, the Hormodendronlike development would appear to constitute a subsidiary phase resulting from a somewhat promiscuous budding process, and hence not to be compared in distinctiveness to the true conidial stage found in nature. The relationship here is comparable, perhaps, to the relationship between the widespread *Cladosporium herbarum* Link and its Hormodendron stage, which was carefully investigated by Bancroft (*1*), although the occurrence of the Hormodendron stage throughout the parasitic life of that fungus to the exclusion of the other is far from having a counterpart in the life history of *Helminthosporium giganteum.* More recently Spangler (*8*) reported the development of Hormodendron fructifications in artificial cultures of *C. fulvum* Cke. and suggested the theory that probably only one type of conidium was produced and that the two-celled bodies usually held presumptive for Cladosporium probably represent nothing but fragments of denuded conidiophores. To the extent to

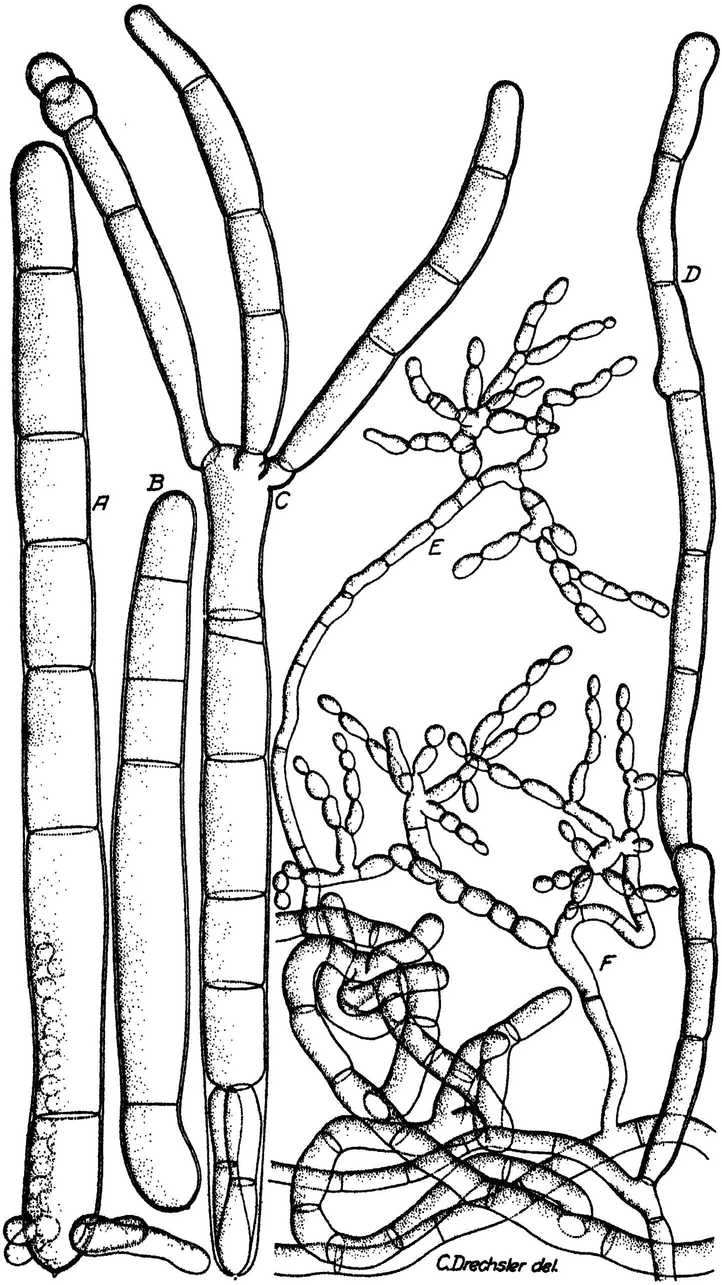

FIG. 2.—A–C, Conidia of *Helminthosporium giganteum* produced on a 20-day-old maize-meal agar culture. A, Normal germination from the basal segment, while an analogous process has given rise in C to three secondary conidia produced from the apical segment. Death of the basal segment in C has resulted in its occupation by hyphal elements arising as "Durchwachsungen" from the adjacent segment. × 450. D, Conidiophore of *H. giganteum* arising from aerial mycelium developed on maize-meal agar. × 450. E and F, Hormodendronlike fructifications of *H. giganteum* arising from aerial mycelium developed on maize-meal agar. × 450

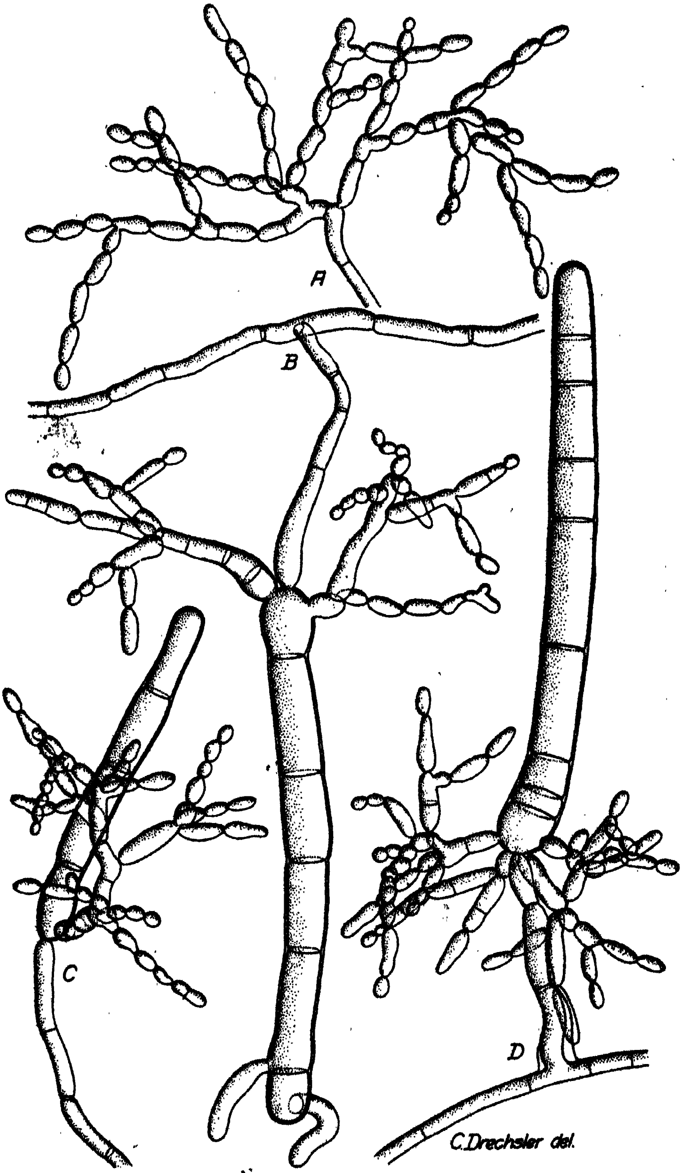

FIG. 3.—A, Hormodendronlike fructification of *Helminthosporium giganteum* arising from aerial mycelium developed on maize-meal agar. × 450. B–D, Hormodendronlike fructifications arising by proliferation from conidia of *H. giganteum* produced on maize-meal agar. × 450

which *H. giganteum* provides a parallelism, it affords little support for this view, but emphasizes rather the departures from normal morphology effected by the conditions of artificial culture. *Hormodendron hordei*, described by Bruhne (*2*) in 1894 as the cause of a leaf spot of barley (*Hordeum vulgare* L.) in Germany, is of interest in this connection as an apparently well-authenticated parasite on a gramineous host. Although the conidia from barley leaves were always warty, they gave rise to smooth spores when cultivated artificially, and the general arrangement of parts shown in Bruhne's figure (*2, Taf. 1, fig. 4*) resembles at least superficially the proliferous condition of the parasite causing zonate eyespot. Because of the presence of septate conidia, Lindau (*7, p. 700–701*) seemed inclined to regard Bruhne's fungus as a stage of Cladosporium, even though the septate structures, as in the case of *C. fulvum*, apparently were not usually terminal, and therefore might equally well have been construed as disarticulated sporophoric segments.

TAXONOMIC RELATIONSHIPS OF PARASITE

The affinities of *Helminthosporium giganteum* remain problematical. As has been pointed out previously (*5*), the large majority of graminicolous species of Helminthosporium are referable to either one or the other of two types, one having typically straight cylindrical conidia germinating indiscriminately from the intermediate as well as from the proximal and distal segments, the other with ellipsoidal conidia germinating normally by the production of two polar germ tubes. Of the species belonging to the former type, several have been identified with ascigerous conditions referable to Pyrenophora or Pleospora, and it would seem probable that a similar affinity will be found to prevail throughout. Several species of the second type have been found connected with a perfect stage, which is represented by a peculiar type of Ophiobolus characterized by helicoid ascospores. *H. giganteum* can not apparently be assigned to either category. While the conidia it produces are cylindrical, their distinctive method of germination by the production of two whorls of three or four germ tubes, one whorl arising at a little distance from the attachment and the other at an approximately equal distance from the apex, is not indicative of any close relation to the forms connected with Pyrenophora. The general appearance of the fungus in artificial culture, its slow rate of growth, the frequent disposition of the aerial mycelium in curiously curving filaments, the Hormodendronlike structure arising from hyphae or, by secondary proliferation, from conidia, the unusually regular contours and homogeneous contents of the submerged hyphae—all these attributes taken together would seem further to set off the fungus from either of the two main categories of Helminthosporium species parasitic on grasses. Although in the production of eyespot lesions the fungus is not greatly different from certain other forms, its more extensive zonate developments presents a pathological effect of striking peculiarity.

Examination of the fungus in collections of field material from different localities and various hosts gives an impression of a high degree of morphological uniformity. The limited number of strains isolated have not revealed any differences sufficiently pronounced to predominate over the rather varied expressions of cultural characters exhibited by individual strains on the same plate culture.

The occurrence of aberrant sectors in such cultures, to which some writers attach much importance, was occasionally observed. (Pl. 8.) In no instance, however, did the variants exhibit a degree of distinctiveness sufficient to make them deserving of special taxonomic consideration.

THE HOST RANGE OF THE FUNGUS

Because of its ability to infect a large variety of hosts, and because of the wide range in degree of pathogenicity expressed, from the production of a barely discernible lesion to the almost complete destruction of the foliage of plants attacked, *Helminthosporium giganteum* might well serve as a subject for inquiry into the intimate aspects of parasitism. Owing to the difficulty of obtaining conidia of the parasite in artificial culture, however, greenhouse experimentation following the usual methods might not be easy of accomplishment. In the absence of such experimentation, field observations on mixed stands of grasses, including one or more species upon which the fungus sporulates abundantly, may not be devoid of interest. Some observations of this kind, together with descriptive data, are presented in the following paragraphs.

Agropyron repens mixed with heavily infected *Cynodon dactylon* was found severely attacked in various localities in the vicinity of the District of Columbia. Equally severe infections were observed, however, in a number of situations where no admixture of Bermuda grass was present, thus supplying proof of a degree of congeniality high enough to permit the fungus to propagate itself luxuriantly independent of other hosts. The individual lesions (pl. 2, I–M) do not generally exceed 1 mm. in width and 3 to 5 mm. in length, although sometimes the latter dimension may approximate 8 mm. They are straw colored in the center and delimited by a narrow dark-brown marginal zone. The zonate type of development usually may be observed, although the destruction of leaves more often is attributable to the abundance of moderate-sized lesions, several hundred of which not infrequently may be present on an individual foliar organ. After the death of severely infected leaves a liberal production of sporophores and spores ensues, the former arising not only from the discolored areas but also from the surrounding tissue.

The abundance of *Agropyron repens*, together with its high degree of susceptibility, seems to indicate that this grass might become the most important host of *Helminthosporium giganteum* in sections near the northern range of the parasite wherever Bermuda grass is present only in lesser quantity.

In August, 1922, at the Arlington Experiment Farm, Rosslyn, Va., *Agropyron elongatum* was found affected with *Helminthosporium giganteum*, although somewhat less severely than *A. repens*. While the infections resulting directly from germinating conidia appeared in considerable number, the lesions remained mostly of small dimensions. (Pl. 2, A.) When secondary enlargement took place and groups of lesions became confluent (pl. 2, B C), more severe effects were brought about. In the same plot another congeneric host, *A. intermedium*, revealed infection of somewhat less severity than that prevailing in quack grass, though otherwise not dissimilar. (Pl. 2, D–H.)

Agrostis stolonifera L., in September, 1922, at Arlington Experiment Farm, showed elliptical spots approximately 1 mm. in width and up to 2 or 3 mm. in length, which were readily attributed to conidia of *Helminthosporium giganteum*. The affected areas, for the most part almost white, were delimited from the healthy tissue by a very narrow, inconspicuous dark-brown margin. (Pl. 2, N–Q.) Owing, apparently, to the small size of the leaves, when a number of lesions occurred on the same blade, withering of the parts more distal in position resulted, although the entire damage caused was not excessive. No extensive zonate development was observed. As the eyespot lesions for the most part remained free from conidiophores of the parasite, it is not evident that the latter is capable of maintaining itself on creeping bent grass.[5]

Bromus inermis was found severely attacked by *Helminthosporium giganteum* at Arlington Experiment Farm during the season of 1922. The number of eyespot lesions, to be sure, was not excessive. In the absence of secondary development they generally did not attain immoderate size, those measuring more than 1 mm. in width and 2 or 3 mm. in length, including the deep-brown delimiting margins, being rather exceptional. (Pl. 3, A–D.) Secondary development, however, was relatively frequent and often extensive. (Pl. 3, D.) On the large regions of killed tissue, sporulation took place abundantly. Undoubtedly awnless brome grass may be regarded as more subject to damage than most of the several cultivated grasses included among the various hosts discussed in the present account. It may be a fortunate circumstance, therefore, that the area over which it is being grown for forage lies well north of the latitudes in which the fungus has hitherto been observed.

Chaetochloa lutescens, although often found growing in proximity to heavily infected Bermuda grass in the vicinity of the District of Columbia during the season of 1922, never exhibited any lesions due to *Helminthosporium giganteum* in any of the collections made in that general locality. That the grass is nevertheless not entirely immune from infection is evident in the occurrence of eyespot lesions in material collected near Hurlock, Md., in August, 1923, as well as in a collection made near Kennett, Mo., in August, 1924. In both cases the source of the infecting conidia was badly diseased Bermuda grass, in a stand of which the yellow foxtail grass occurred as an intimate admixture. The lesions, so few in number as almost to escape detection, were of relatively small size, not usually exceeding 1 mm. in length and 0.5 mm. in width, and of an elliptical shape, with a straw-colored central portion surrounded by a brownish margin. (Pl. 3, E, F.) As might be expected, conidiophores of the parasite were never observed on any of the spots.

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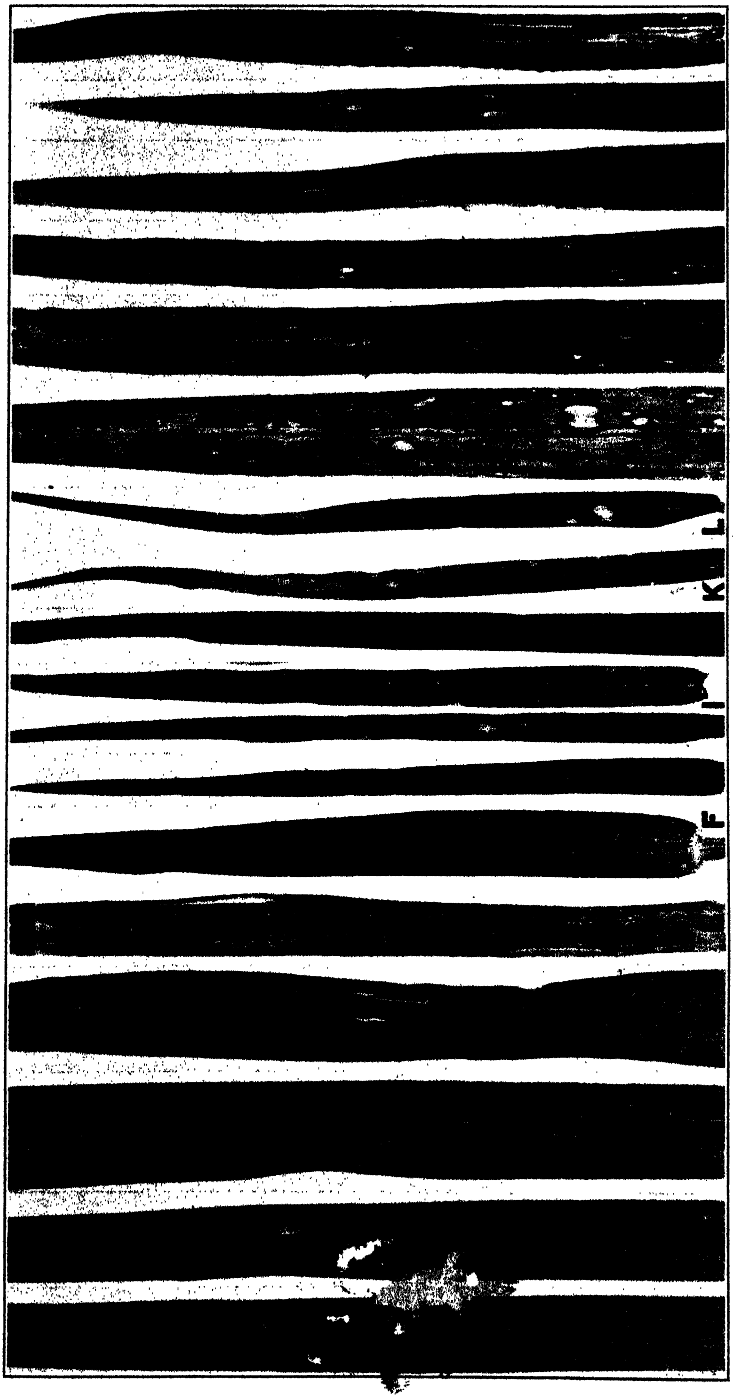
[5] Severe infection of creeping bent was noted in some of the turf plots and nursery rows at the Arlington Experiment Farm during the season of 1928, the very evident destructiveness of the parasite even in the absence of artificial watering being associated with ready centrifugal development of lesions and abundant sporulation. The position of the grass under consideration as an independent host was confirmed in a striking way by the occurrence of zonate eyespot in putting greens planted with it. This was true not only in regions generally favorable for the development of the fungus, but also, as has been mentioned in another connection, in territory not known to harbor the parasite on any host under ordinary conditions. In the vicinity of La Fayette, Ind., greens visited by the writer on Aug. 28, 1928, showed heavy infection, though perhaps owing to cooler conditions the infection then was less severe than that represented in specimens collected from the same grounds on July 20, 1928, by A. A. Hansen. Golf courses in the vicinity of Detroit, Mich., visited Sept. 1, 1928, showed the parasite active on some greens, though only in moderate or even small quantity; and a similar degree of prevalence was found also at Wooster, Ohio, visited Sept. 4, 1928. Specimens originating from near London, Ohio, from near De Kalb, Ill., from near Highland Park, Ill., and from near Minneapolis, Minn., in August and September, 1928, provide additional testimony of the efficacy and wide distribution of *Helminthosporium giganteum* as a turf parasite. It may be mentioned that not all strains of creeping bent are attacked with equal severity, some strains appearing almost completely resistant. Indeed the meager infection observed at the Arlington Experiment Farm in 1922 is to be explained by the fact that during that season only resistant types were represented in the nursery rows, whereas the destructive infection recorded for 1928 was limited to one or several very susceptible types subsequently added to the plantings.

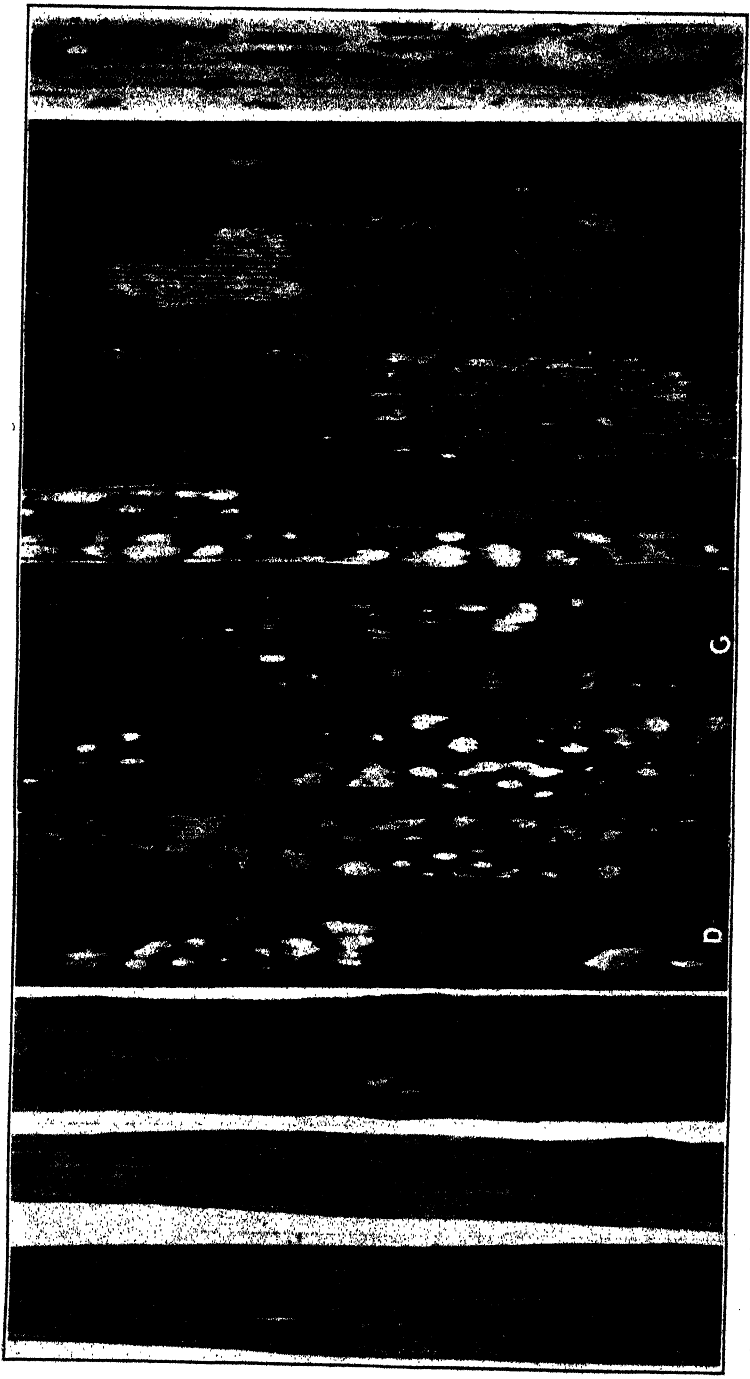
Cynodon dactylon unquestionably serves as the principal host of *Helminthosporium giganteum* in the United States. Infection by germinating conidia evidently takes place with unusual readiness, the foliage usually becoming spotted with lesions of independent origin much more abundantly than is shown in Plate 3, G–I. When conditions are favorable, the secondary type of development occurs (pl. 3, J–L) in about the same measure as in *Agropyron repens* and *A. intermedium*, and therefore perhaps somewhat less extensively than in *Phalaris arundinacea* or even in *Bromus inermis* and *Eleusine indica*. Nevertheless, because of its widespread distribution throughout at least the more favorable range of the fungus, and the readiness with which sporulation proceeds on the diseased foliage, mostly on tissue involved in the coalescence of groups of crowded individual lesions, Bermuda grass appears, on the whole, to support the parasite in as large quantity as all the other hosts taken together. The densely massed habit it frequently adopts when left undisturbed on suitable soil seems to be unusually favorable for the development of the fungus, so that the more luxuriant stands are frequently all but killed outright, the severity of such attack not being exceeded by any foliar grass disease known to the writer.

Digitaria humifusa Pers. (*Syntherisma ischaenum* Schrad. Nash), growing mixed with heavily infected Bermuda grass at Kennett, Mo., in August, 1924, bore a liberal sprinkling of lesions due to *Helminthosporium giganteum*. These lesions (pl. 3, M–O) occurred as elliptical spots rarely exceeding 2 mm. in length and 1 mm. in width, and having a straw-colored center with a reddish brown delimiting margin. The zonate type of development never was manifested. Examination of the eyespot lesions failed to reveal any conidiophores of the parasite in question. In the same location *Digitaria sanguinalis* bore eyespot lesions caused by germinating conidia of *H. giganteum*, resembling those borne on the congeneric host, but exhibiting a somewhat broader, more deeply colored border, and occurring so sparingly that their discovery entailed considerable search. The leaf shown in Plate 3, Q, was very unusual, in that nearly a dozen spots were found relatively close together, while that shown in Plate 3, R, with only two, also represents a more heavily infected condition than obtained generally. No conidiophores were found on any of the lesions on crabgrass.

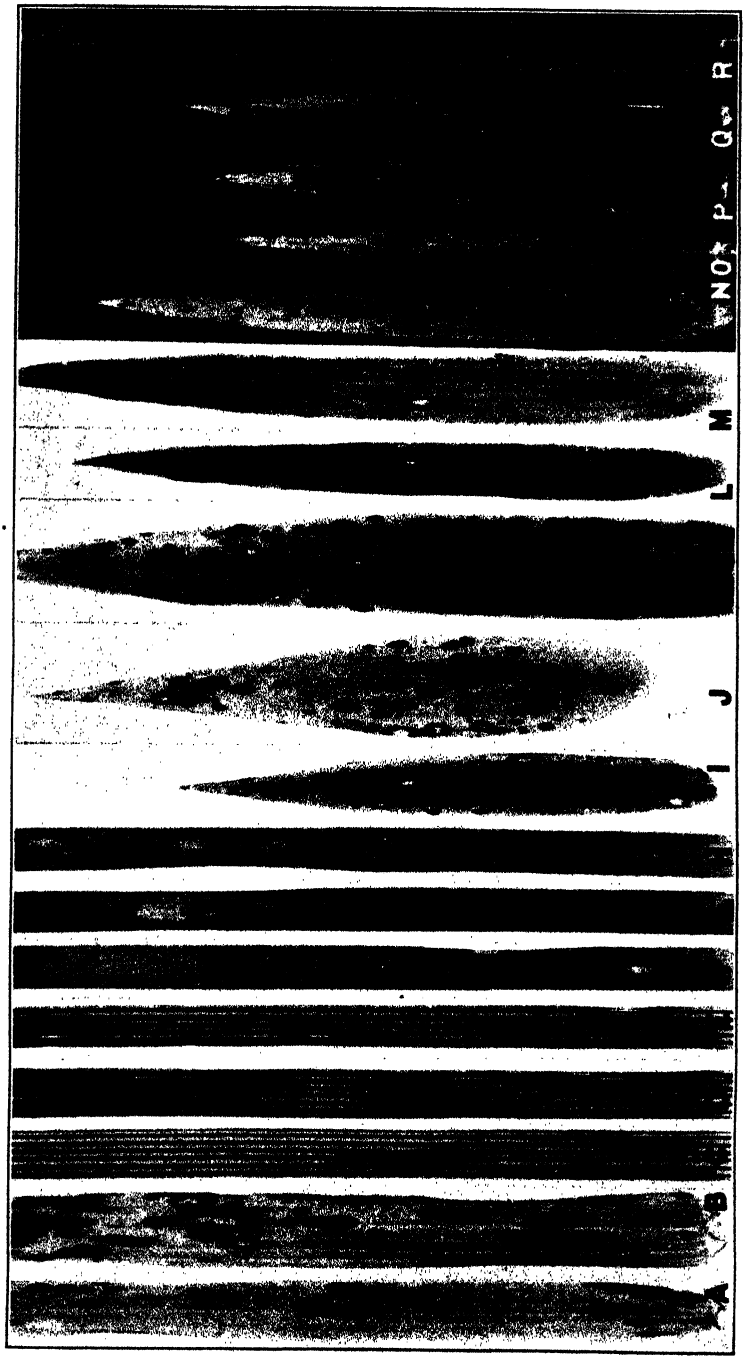
Echinochloa crusgalli was found attacked by *Helminthosporium giganteum* near Kennett, Mo., in August, 1924. It exhibited a considerable degree of susceptibility, the individual lesions being not only fairly numerous but also often showing moderately extensive secondary development. (Pl. 4, A–C.) Reddish brown coloration was somewhat conspicuous, being present on the relatively broad margins delimiting the discrete lesions, as well as in larger blotches encompassing areas killed as a result of secondary development, or resulting from coalescence of [illegible] of separate spots. On the larger withered parts sporophores and spores were being produced in quantity. Barnyard grass would seem to show sufficient congeniality to serve as one of the more important hosts, although in the localities



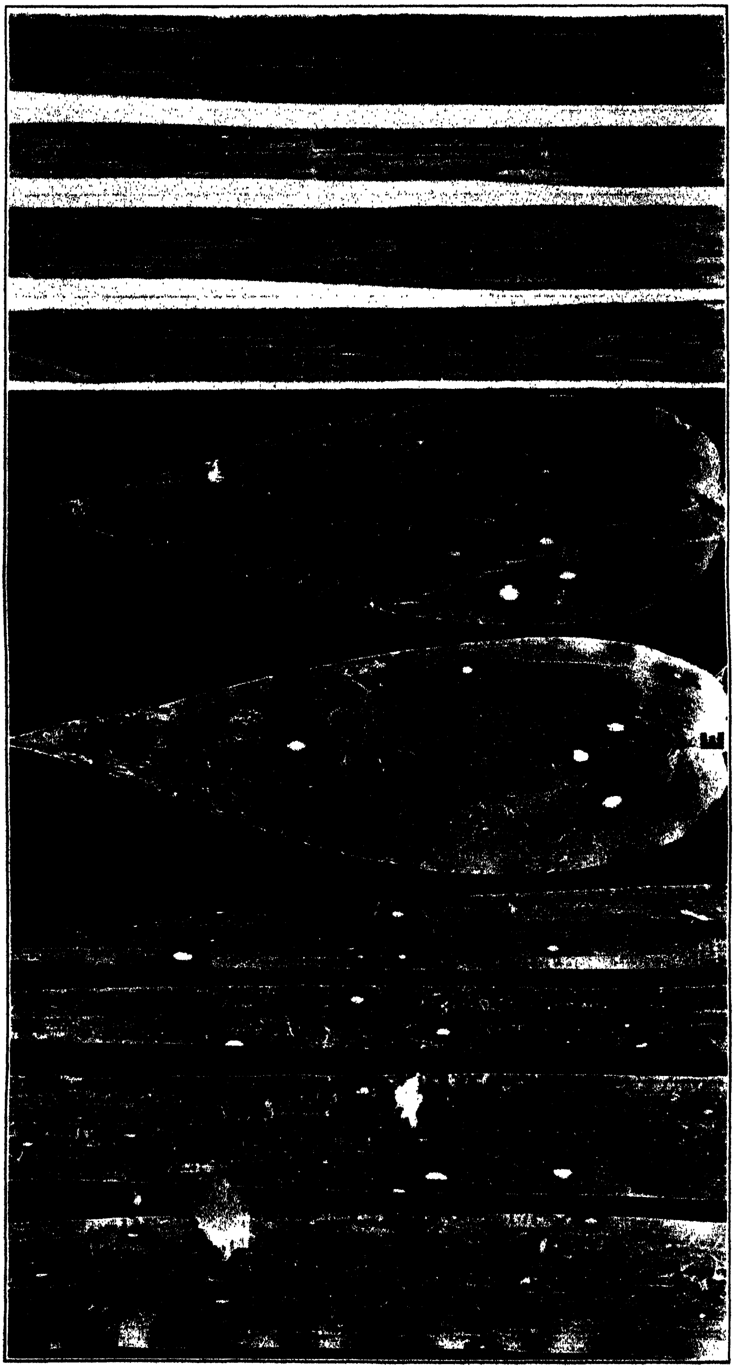
Leaves of various grasses attacked by *Helminthosporium giganteum*. A–D, *Bromus inermis*; E, F, *Chaetochloa lutescens*; G–L, *Cynodon dactylon*; M–O, *Digitaria humifusa*; P–R, *Digitaria sanguinalis*. × 2



Leaves of various grasses attacked by *Helminthosporium giganteum.* A–C, *Echinochloa crusgalli;* D–H, *Eleusine indica;* I, J, *Elymus virginicus;* K, *Eragrostis major* × 2



Leaves of various grasses attacked by *Helminthosporium giganteum.* A, B, *Eragrostis major;* C-H, *Lasiagrostis splendens;* I-K, *Leersia virginica;* L, M, *Muhlenbergia mexicana;* N-R, *M. schreberi.* × 2



Leaves of various grasses attacked by *Helminthosporium giganteum.* A–D, *Panicum anceps;* E, F, *P. clandestinum;* G–J, *P. dichotomoflorum.* × 2 in which the writer made his observations it did not occur abundantly enough to play any large part in the maintenance of the parasite.

Eleusine indica is to be included among the grasses most susceptible to *Helminthosporium giganteum*. In infected stands lesions originating from separate infections may become so numerous as to coalesce and thus lead to the withering of individual leaves, or considerable portions of a leaf may be killed directly as a result of secondary enlargement of some of the lesions. (Pl. 4, D–H.) Reddish brown coloration is present in the narrow marginal zones delimiting the individual lesions and also in less sharply localized form in markings on the larger affected parts. Sporulation under suitable conditions is abundant. Goose grass has been found more or less seriously affected wherever the parasite has been encountered. Owing to its general distribution throughout the known range of the parasite, its importance as a host of *H. giganteum* would seem second only to Bermuda grass. Like the latter, it frequently serves as the source from which other grasses in close proximity become infected.

Elymus virginicus, growing at Arlington Experiment Farm within 10 meters of diseased Bermuda grass in the season of 1922, showed a somewhat unusual condition relative to its infection by *Helminthosporium giganteum*. The individual lesions resulting directly from germinating conidia were few in number, but a large proportion of these showed extensive secondary development. (Pl. 4, I, J.) On the zonate areas of killed tissue sporophores were produced abundantly. The appearance suggested that infection of the coarse foliage by germinating conidia was attended with difficulty, but that once the parasite gained a foothold its further development centripetally took place readily. Under suitable conditions the grass would seem to be capable of serving as a congenial host.

Eragrostis major, growing in mixed stand with very heavily infected Bermuda grass near Seat Pleasant, Md., in September, 1922, exhibited meager infection by *Helminthosporium giganteum*. The large majority of lesions were of the eyespot type, elliptical in shape, from 0.2 to 0.8 mm. in width and 0.4 to 1.6 mm. in length, with a rather conspicuous deep reddish brown marginal zone surrounding a central bleached area usually of minute size. (Pl. 4, K; 5, A, B.) In a number of instances, however, secondary development had resulted in the death of more extensive portions of tissue measuring sometimes from 10 to 20 mm. in length and from 2 to 3 mm. in width, or even extending entirely across the leaf. Withering of the distal portions of certain foliar organs in some cases appeared to result from such more extensive development of the parasite, or from an unusual concentration of smaller lesions, although a certain degree of doubt as to the causal relation of the parasite was introduced because of the approaching maturity of the host. Sporophores of the fungus were found on the larger lesions and on withered parts bearing numbers of smaller spots in close proximity to one another. Such reproduction, however, was on a decidedly small scale, and it remains somewhat uncertain, therefore, whether the fungus could propagate itself successfully on stink grass alone.

Lasiagrostis splendens, growing at some distance from heavily infected reed canary grass at Arlington Experiment Farm in 1922, became severely infected with *Helminthosporium giganteum*. Individual lesions were fairly numerous, the smallest ones appearing as uniformly dark-brown blotches. (Pl. 5, C. D.) Those of intermediate size, measuring 2 to 4 mm. in length, were generally of the simple eyespot type (pl. 5, E. F), while the more extensive morbid areas, frequently exceeding 1 cm. in length and including the entire width of the leaf, bore the zonate markings characteristic of secondary development. (Pl. 5, G, H.) On the latter type of lesion sporophores and spores of the parasite were found occurring in abundance. Because of the strong dorsiventral differentiation between the prominently veined dark-green upper surface of the foliage (pl. 5, C–E) and the smoother, lighter green under surface (pl. 5, F–H) the two aspects of the lesions appear different to a rather unusual degree. The fungus would seem capable under suitable conditions of causing more than appreciable injury to the grass and unquestionably could maintain itself thereon independent of other hosts.

Stands of *Leersia virginica*, occurring in close proximity to heavily infected Bermuda grass or quack grass at various points along the Chesapeake & Ohio Canal, have regularly become thickly peppered with numerous eyespot lesions during the five seasons in which observations were continued. While the spots usually remain relatively small, rarely exceeding 2 mm. in length and 1 mm. in width (pl. 5, I–K), they occasionally become confluent, and thus bring about the death of somewhat larger portions of leaf. Even these larger areas, however, are usually devoid of conidiophores of *Helminthosporium giganteum*, although in somewhat exceptional instances a very sparse array of such structures has been observed. That such meager sporulation, nevertheless, is not entirely without significance became evident through the discovery in September, 1922, of a pure solitary stand of white rice grass on which an infection with *H. giganteum* occurred obviously quite independent of other hosts. As this stand was situated on a large fill on which other grasses had not encroached, it was not difficult to verify the absence of external sources of infection within a radius of more than 50 meters. It is interesting to note that extensive secondary development of the fungus from relatively few lesions, rarely observed elsewhere, here accounted largely for the injury observed, which, to be sure, was inconsiderable. Sporulation on the larger tracts of leaf tissue involved in such development was only slightly more abundant than on the leaves bearing the numerous small lesions of independent origin. The grass is to be regarded, perhaps, as hardly a more congenial host than *Eragrostis major*, even though under exceptional conditions it permits autonomous propagation of the parasite.

Sometimes *Leersia virginica* can be found attacked simultaneously by both *Helminthosporium giganteum* and *H. leersii* Atk. As the older lesions caused by the latter fungus are many times larger than eyespot lesions due to the former, and never exhibit the zonate markings characteristic of the secondary development of *H. giganteum*, their identification usually entails little trouble. The smaller lesions of *H. leersii*, also, can generally be distinguished from those of *H. giganteum* because of their broader and less sharply defined marginal zone. In doubtful instances microscopic examination is necessary. Since neither fungus sporulates on any except the largest regions of affected tissue, identification of smaller spots is most conveniently accomplished by determining the presence or absence of the evacuated spore membrane of *H. giganteum.*

Muhlenbergia mexicana, at a distance of about 5 meters from heavily infected quack grass, revealed relatively few scattering lesions of *Helminthosporium giganteum.* The larger ones were elongated elliptical in shape, measuring 2 to 3 mm. in length by 0.5 mm. in width, and showing sharp differentiation between the small central bleached portion and the narrow dark-brown delimiting zone. (Pl. 5, L, M.) Most of the lesions were of the unmodified eyespot type and quite devoid of sporophores of the parasite. Occasionally the presence of minute specklike discolorations in zonal arrangement about one of the larger lesions evidenced a somewhat meager secondary development. In certain of the largest lesions sporophores of the fungus were observed, although the total production of such structures was so small that it is to be doubted whether the fungus could persist on *Muhlenbergia mexicana* in the absence of more favorable hosts, except under very favorable conditions.

Muhlenbergia schreberi, in the same locality as *M. mexicana* but occurring in intimately mixed stand with heavily infected quack grass and goose grass, exhibited lesions of *Helminthosporium giganteum* in moderate number. These lesions were characterized by unusually small size, sharp definition of the margin from the healthy tissue and the bleaching of the center in spite of relatively minute proportions. (Pl. 5, N–R.) No extensive secondary development or evidence of sporulation ever was observed on this grass, which evidently does not permit autonomous development of the fungus. Owing to the frequent occurrence, on the more mature foliage, of numerous minute dark linear lesions somewhat resembling those due to conidia of the parasite under consideration, but associated with another fungus, spots not bleached in the center can not be identified without microscopic inspection.

Panicum anceps Michx., occurring interspersed in a stand of heavily infected quack grass during the season of 1922, exhibited eyespot lesions in moderate quantity. These lesions sometimes attained a length of 3 to 4 mm. and a width of 1.5 to 2 mm., although usually their proportions did not exceed one-half of the values mentioned. (Pl. 6, A–D.) They exhibited a bleached center on attaining a length of 1 mm., the delimiting margin being usually relatively broad and light brown in coloration, rather than dark brown. As none of the lesions were found bearing conidiophores of *Helminthosporium giganteum*, the grass can not be considered among the congenial hosts.

Panicum clandestinum, because of its habit of occupying the weedy borders of neglected fields, which, after the middle of summer in the vicinity of the District of Columbia, are often overrun with Bermuda grass, was frequently found exposed to infection from the great profusion of conidia produced by *Helminthosporium giganteum* on the latter host. The foliage then exhibited discoloration in the form of numerous dark-brown specks, or of larger nebulous blotches, or of well-defined eyespot figures, with a relatively wide, vaguely delimited marginal zone. (Pl. 6, E–F.) When the younger leaves thus affected were examined microscopically, these discolored portions could in all instances be found associated with collapsed remains of overlying conidia of the parasite. Although the larger eyespot lesions contained bleached central areas that sometimes measured 4 mm. in length by 1.5 mm. in width, sporophores of the fungus never were observed. In spite of a relatively high degree of susceptibility to conidial infection, the grass is apparently not sufficiently congenial as a host to sustain *H. giganteum* independently.

Pancium dichotomoflorum was found intermixed with infected goose grass in a number of truck fields and vegetable gardens near the District of Columbia in September, 1922. Many of the leaves thus exposed bore a varying number of lesions from infection by conidia of *Helminthosporium giganteum*. Near Kennett, Mo., where, in September, 1924, the grass occurred in mixed stand with heavily infected Bermuda grass, a considerably more abundant infection obtained. In both localities the lesions were represented by reddish-brown or dark-brown spots, rather sharply defined from the healthy tissue, somewhat linear or streaklike in outline, variable in size, often being so small as to be barely discernible, but sometimes attaining a length of 3 mm. and a width of 1 mm. (Pl. 6, G–J.) Many of the lesions were bleached in the center, but in other instances this feature was not evident. None of the material from either source revealed the presence of sporophores.

Panicum gattingeri, growing in a stand of infected quack grass at Cabin John, Md., in September, 1922, bore a meager sprinkling of eyespot lesions due to infection from conidia of *Helminthosporium giganteum*. The spots were small in size, being not more than 1 mm. long and less than half as wide, yet usually exhibiting a bleached center. (Pl. 7, A–C.) Conidiophores of the fungus never were observed. The grass is evidently considerably less susceptible to infection than any of the three congeneric species mentioned, and is to be included among the more unfavorable hosts.

Pennisetum alopecuroides (L.) Spreng.,[6] growing at a distance of less than 1 meter from heavily infected reed canary grass at Arlington Experiment Farm in 1922, showed on some leaves scattered lesions due to infection from conidia of *Helminthosporium giganteum*. These lesions were present generally as reddish brown blotches, although a few were of the eyespot type, with the bleached center sharply defined. (Pl. 7, D–G.) None ever revealed the presence of conidiophores. In view of the quantity of inoculum to which the foliage was exposed throughout the season and the insignificance of the injury occasioned, the grass would appear to possess little susceptibility to attack by the fungus.

The extreme congeniality of *Phalaris arundinacea* as a host of *Helminthosporium giganteum* has been discussed in another connection. It must be mentioned, however, that the parasite has not been encountered on reed canary grass elsewhere than in the plots at Arlington Experiment Farm. Several wild stands observed in the vicinity of the District of Columbia never revealed any sign of infection, even during the very favorable season of 1922. As all of these stands have happened to occur in isolated situations, separated from infected grasses by wooded terrain, the absence of the fungus was not difficult to explain. Nor have infections ever been observed

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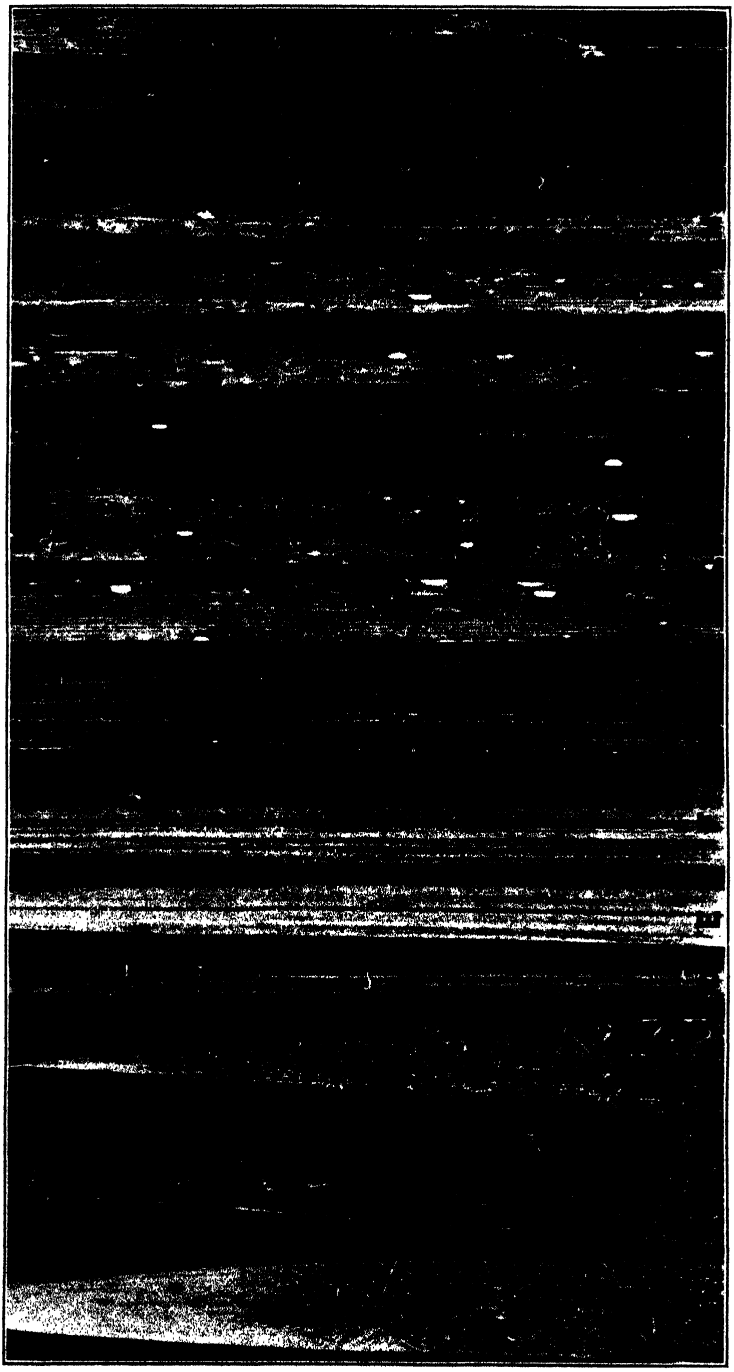
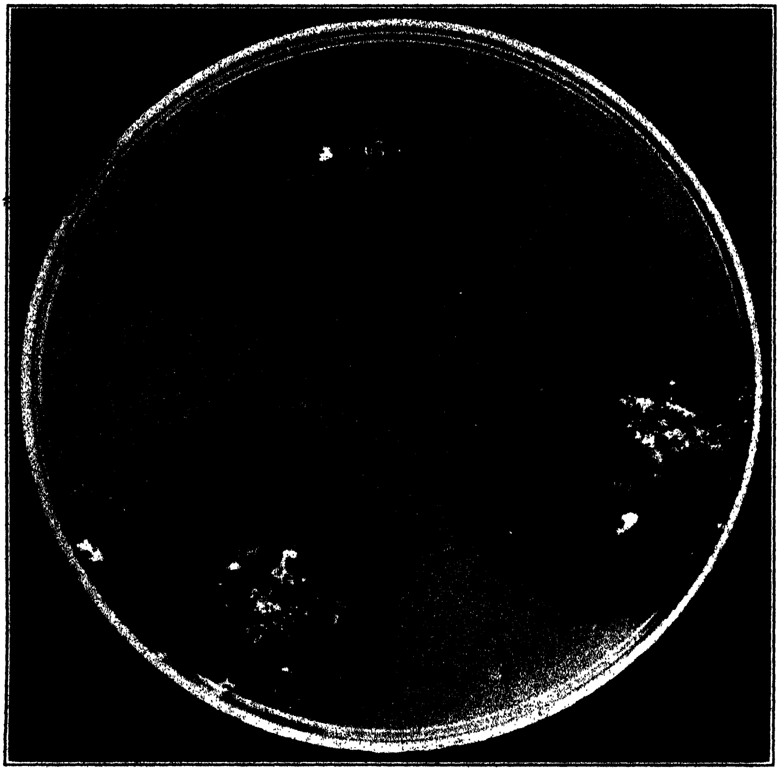
[6] This host was reported previously under the binomial *Pennisetum japonicum* Trin. The change in specific name is made on the advice of Agnes Chase, who kindly examined specimens and identified them as clearly belonging to the species frequently designated as *P. compressum* R. Br. The plant is of course conspicuously different from pearl millet (*P. glaucum* (L.) R. Br.), to which it would appear the term *alopecuroides* during a long period of nomenclatorial confusion has often been erroneously applied.



Leaves of various grasses attacked by *Helminthosporium giganteum*. A-C, *Panicum gattingeri*; D-G, *Pennisetum alopecuroides*; H-L, *Phleum pratense*; M-P, *Poa pratensis*. ×2



Maize-meal agar plate culture of *Helminthosporium giganteum* 15 days after inoculation. × 1 on the ornamental form known as ribbon grass, *Phalaris arundinacea* var. *picta* L.

During several seasons *Phleum pratense*, found growing among heavily infected Bermuda grass and quack grass in a number of locations near the District of Columbia, exhibited, in meager number, lesions caused by germinating conidia of *Helminthosporium giganteum*. These lesions usually were relatively small, rarely exceeding 2 mm. in length or 1 mm. in width. (Pl. 7, H–L.) Often they consisted only of bleached portions of tissue, thus appearing as white spots, devoid of any colored margin, while in other cases a narrow brown border was recognizable. The scarcity of infection and the complete absence of conidiophores from the relatively small diseased parts denote a high degree of resistance to the parasite.

Poa pratensis, often found exposed to infection in the same situations as timothy, exhibited approximately the same low degree of suceptibility. The lesions were similarly few in number and of equally small dimensions, some, indeed, being so minute as to be barely discernible. (Pl. 7, M–P.) A conspicuous difference was represented in the dark-brown or brownish-black color of the spots on Kentucky bluegrass. The bleached center distinctive of the eyespot lesions was present only in exceptional instances, and then somewhat vaguely, most of the discoloration appearing in the form of unrelieved elongated or almost linear specks.

SUMMARY

Helminthosporium giganteum occurs generally throughout the Southern States and has been found in quantity as far north as Maryland and Missouri. In the vicinity of the District of Columbia the conidia from centers of infection do not appear to spread beyond distances of 20 meters in one season, a limitation due apparently to the large size and short period of viability of these structures, and to which may be attributed, in part at least, the frequent irregularities noticeable in the local distribution of the parasite. The fungus seems to overwinter in the form of dormant mycelium, fresh conidiophores and conidia being produced in late spring from the morbid parts of old foliage infected during the previous season.

Sporulation of the fungus occurs on the larger tracts of killed tissue, resulting either from the coalescence of numbers of individual eyespot lesions or from secondary development of such lesions. The latter type of development occurs only when the leaf surface is coated with moisture, and involves the production, centrifugally from the margin of the morbid tissue, of a prostrate mycelium that in occupying the surrounding parts brings about a water-soaked condition and later desiccation and death. As in various hosts the newly infected parts are delimited by marginal coloration, the repetition of such development brings about a characteristic zonate appearance.

Among the hosts on which sporulation was observed under natural conditions, and on which the parasite apparently could propagate itself, are to be included *Agropyron elongatum*, *A. intermedium*, *A. repens*, *Bromus inermis*, *Cynodon dactylon*, *Eleusine indica*, *Echinochloa crusgalli*, *Elymus virginicus*, *Lasiagrostis splendens*, *Leersia virginica*, and *Phalaris arundinacea*. Sporulation was observed also, though in meager quantity, on *Eragrostis major* and *Muhlenbergia mexicana*. Lesions due to infection by conidia of the parasite were observed on *Argostis stolonifera, Chaetochloa lutescens, Digitaria humifusa, Muhlenbergia schreberi, Panicum anceps, P. clandestinum, P. dichotomoflorum, P. gattingeri, Pennisetum alopecuroides, Phleum pratense,* and *Poa pratensis* when these grasses occurred in proximity to more congenial hosts.

When grown in pure culture on artificial media the fungus develops relatively slowly. Meager and somewhat abnormal sporulation generally takes place, the conidia as well as the mycelial hyphae often giving rise to branching systems of small disarticulating elements, the whole closely resembling the fructifications usually referred to the form genus Hormodendron. This proliferous phase, and more especially the distinctive method of germination by the production of two whorls of germ tubes, one from near each end of the conidium, would seem to set the fungus apart from the two most numerous series of graminicolous forms included in the genus Helminthosporium.

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STUDIES ON FIRE BLIGHT: HOST RANGE[1]

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INTRODUCTION

For the past 50 years or more the disease of rosaceous plants popularly known as fire blight has been the subject of innumerable studies, mainly by American plant pathologists. Over 200 articles on fire blight have recently been reviewed by the junior author (*10*),[2] every one of which added some facts that had not been known prior to its publication. Hundreds of other articles and bulletins on the same subject were excluded from this list because they represented no new work and were largely written for popular reading. In spite of this impressive array of literature it can hardly be said that practical and economical measures for the control of this disease have yet been devised. Aside from the practical aspects, many fundamentally important scientific facts are wanting. Even the morphology of the pathogene, a question which is apt to receive the first attention in the study of any parasitic disease, has not been fully established. Discussions of cultural and physiological reactions of the pathogene are often conflicting and wanting in exact details, many of them having been written at a time when the science of bacteriology was very young (*3*), and when pure cultures were frequently not obtained, owing to the imperfect and laborious technic then available. Does the organism overwinter only in very susceptible varieties and species or is it free from such limitations? Why are plants extremely susceptible in one month and very resistant in the month following? By what means does the organism produce the disease? These questions have not received adequate attention.

THE RANGE OF HOSTS

From the standpoint of parasitism in general and cellular pathology in particular, the disease producer here involved offers some exceptionally tangible points of investigation. Very few bacterial pathogenes are capable of attacking such a wide range of genera, species, and varieties of plants. Two new genera and several new species of hosts will here be presented, and there can be little doubt that others remain to be discovered. Now, while most of the bacterial pathogenes capable of attacking a wide range of hosts are primarily wound parasites, such as *Bacterium tumefaciens* and *Bacillus carotovorus*, *B. amylovorus*, on the other hand, is fully capable of entering through at least one kind of natural opening, the nectary, and perhaps under certain conditions, if we accept Heald's work (*11*), it may also enter through the hydathodes. (Brooks's recent investigations (*6*) seems to throw some doubt on the last possibility, and there can be little

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[1] Received for publication July 21, 1928; issued November, 1928. Research paper No. 9, Journal series, University of Arkansas.

[2] Reference is made by number (italic) to "Literature cited," p. 504.

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doubt that aside from blossom infection, most, if not all, of the others, including those on leaves, fruits, twigs, large limbs, and trunks, as well as roots, involve infections through wounds.) Here then is a pathogene capable of infecting through natural openings as well as through wounds and at the same time possessing a rather wide host range. These facts, as already intimated, open up certain interesting possibilities concerning parasitism. If chemotaxy plays any rôle at all in initiating infections in this disease it must be dependent upon one or more substances which are common to all the hosts involved. Then again the process involved in the production of plasmolysis, cellular discoloration, necrosis, and disintegration by this organism, in brief the manner in which it produces disease on all the hosts, can not easily be looked upon as due to any one toxic substance eleborated by the parasite which possesses any marked degree of specificity but may more reasonably be sought in some general process in which normal cellular functions are interfered with. Aside from these questions engendered by a knowledge of the proper host range, it is very important to know all the susceptible species of plants because of the part which any one plant may have in carrying the disease producer through the winter or because such plants may be important disseminating centers during the growing season.

ARTIFICIAL INFECTIONS ON JAPANESE QUINCE

While many references can be found in the literature to natural and artificial infections on the cultivated quince, *Cydonia oblonga* Mill. (*C. vulgaris* Pers., *Pyrus cydonia* L.), no reference has been found to natural or artificial infections on the flowering quince, *Chaenomeles lagenaria* Koidz. (*C. japonica* Hort., *Cydonia japonica* Hort.), a shrub very commonly used for ornamental purposes over a large part of the United States. As this plant is considered to be closely related to the quince, being placed in the genus Cydonia by some taxonomists and in the closely related genus Chaenomeles by others, it seemed desirable to ascertain its susceptibility to the fire-blight organism. In a preliminary experiment begun on March 29, 1928, a number of blossoms attached to a flowering quince plant growing out of doors were sprayed with a pure culture of *Bacillus amylovorus* which had been isolated from a blighted apple twig and which when inoculated into potted Bartlett pear plants growing in a greenhouse had proved to be very virulent. By April 10 a few of the inoculated blossoms showed various signs of blight, including a withering and discoloration of the petals and a dark green water-soaked appearance of the receptacles. Microscopic examination of the receptacles showed the tissues to be teeming with bacteria of a size and shape which clearly indicated the fire-blight bacillus. Uninoculated blossoms appeared perfectly sound and without any evidence of infection. As it is difficult to maintain proper controls out of doors it was decided to repeat the experiment in the greenhouse in a more adequate fashion. A number of shoots were cut off, some bearing blossoms and others representing newly developed leafy twigs, and placed with their cut ends in vessels containing water.

On April 10, 59 blossoms were inoculated, 31 by spraying with a broth suspension of the strain previously mentioned and 28 by injecting the receptacles with a hypodermic needle. The control consisted of 45 blossoms borne on twigs which were kept apart from those inoculated with the bacterium. Of this number 18 were injected with sterile water by means of a hypodermic needle and 27 were sprayed with sterile water. Three days later a considerable number of the inoculated blooms showed clear signs of blight similar to those noted in the out-of-door inoculations. On April 19, 26 of the 28 plants inoculated with the needle had blighted and 26 of the 31 sprayed plants showed unmistakable symptoms of blight. (See fig. 1.) All the control blossoms remained healthy. In spite of the fact that the blooms blighted so readily, in no case was the disease found to extend into the older tissues of the subtending twigs, being entirely confined to the blooms, and giving them the appearance of having been injured by frost. Indeed the resemblance to frost injury is so striking that it would be very difficult to distinguish one from the other without microscopic examination. Typical blight-

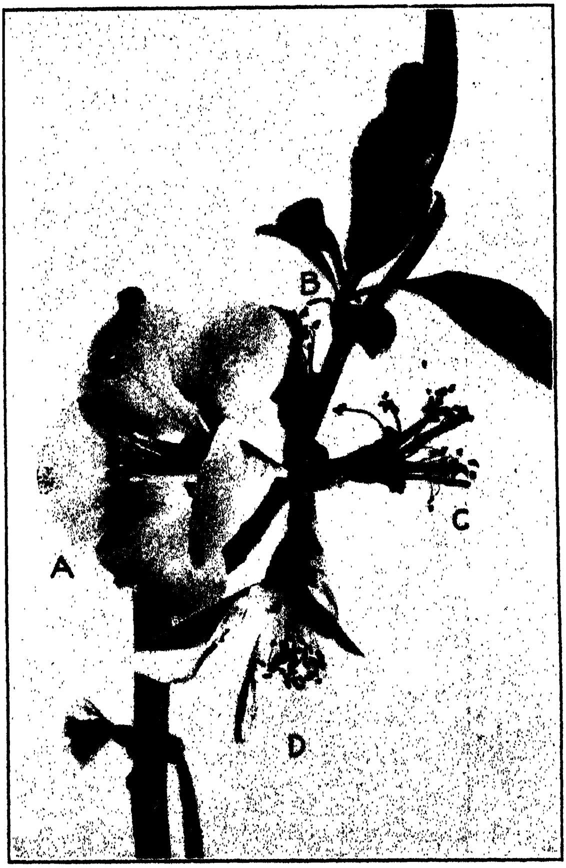

FIG. 1.—Artificial infections on Japanese quince blossoms: B and C, Blossoms sprayed with a pure culture of *Bacillus amylovorus;* A and D, controls. Photographed 10 days after the inoculations were made. Note the withered and discolored appearance, as well as the loss of petals, of the inoculated blooms

producing bacteria were again observed in the diseased blossoms, being found in the petals, calyx, receptacle, and ovary. One of these blighted blooms was carefully washed, the surface sterilized with mercuric chloride, macerated, and used for a series of poured plates. From these the organism was recovered in pure culture and when inoculated into healthy pear shoots of vigorously growing Bartlett plants maintained in the greenhouse, typical blight was produced.

Since no twig blight had developed as a result of the blossom infections, and since the blossoms in this plant are carried on old wood, it seemed worth while to determine the susceptibility to the disease of young, newly developed, leafy shoots. For this purpose a number of young and succulent twigs were inoculated hypodermically and a similar number, used as controls, were injected with sterile water. Here, as in the blossom inoculations, infections occurred very readily. (See fig. 2.) Within a week after the inoculation the disease extended along several inches of the twig, causing it to wither and become dark brown and droopy, killing the attached leaves. Altogether, the twig infections appeared very similar to those observed in ordinary blight of apply twigs, except that the injured and dead twigs showed a greater tendency to droop than do blighted apple twigs. The disease, having shown a progressive killing of adjoining tissues for about 15 days following the first symptoms, soon ceased to extend any further and became sharply delimited by a well-defined margin which frequently appeared depressed in contrast to the adjoining healthy tissues. The dead leaves showed the same tendency to hanging on to the twigs for considerable periods that is often manifested by other hosts. The microscopic observations of the diseased twig tissues revealed the typical blight-producing organisms, and the reisolations, carried out as in the artificial infections of the blossoms, resulted in pure cultures of virulent, blight-producing bacteria, as proved by inoculations on growing pear shoots. The controls all remained sound and free from any signs of blight.

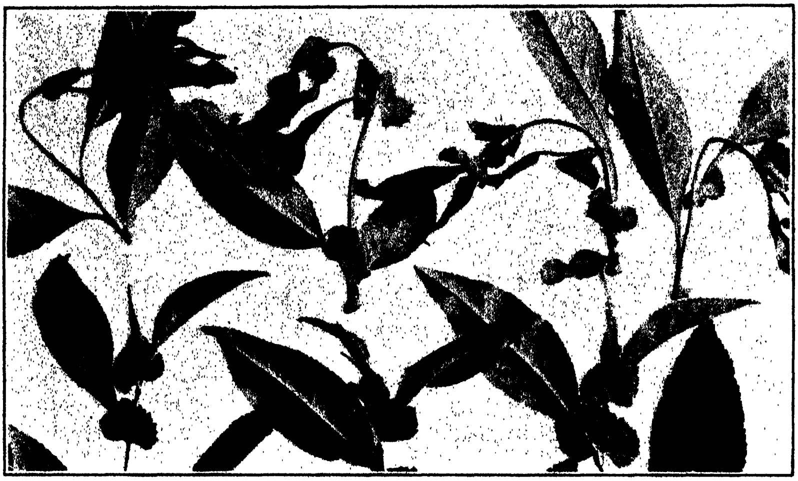

FIG. 2.—Artificial infections on Japanese quince twigs. The four upper shoots were inoculated with a pure culture by the use of a hypodermic needle. The lower shoots, serving as controls were injected with sterile water. Photographed eight days after the inoculations were made

The ease with which artificial infections may be produced on the flowering quince, especially on the blossoms, is quite interesting. No effort whatever was made in these inoculation experiments, either in the sprays or in the injections, to prevent drying out of the inoculum, the inoculated parts being permitted to dry out naturally in the field and in the greenhouse. In spite of this, over 85 per cent of the inoculations were successful, and there is no reason to doubt that this percentage would have been greater had an effort been made to keep the twigs in a saturated atmosphere. In view of these facts why have no natural infections been found on this host and is there any good reason for believing that it is not subject to infection under the usual conditions? The only thing known to the writers that may conceivably interfere with blossom infection in this host is the earliness of its blooming period, but even this does not appear as a valid objection because some of the pear varieties, such as the Kieffer, bloom about the same time in the neighborhood of Fayetteville, Ark. On the other hand, there are excellent reasons for believing that where there is fire-blight inoculum which may be distributed to flowering quince blossoms (and bees, flies, ants, aphids, and other insects appear commonly in and around these), infections are apt to occur. It is quite possible that infections have been overlooked in the past because of the striking resemblance of blossom blight to frost injury and because of the fact that twig as well as blossom infections are inconspicuous, involving very little tissue. However this may be, there are very good indications that the disease may not be expected to do serious injury to the twigs of this host and that the blossoms are the only parts that suffer seriously.

ARTIFICIAL INFECTIONS ON THE ROSE

In 1925 Waite (*25*) succeeded in artificially infecting mature winter apples and rose cuttings by placing them under "forced conditions of the damp chamber, or bell jar." He placed three sets of three rose cuttings, whose lower ends were immersed in water, under bell jars lined with moistened filter paper. On the slanting surfaces of the free ends of two of these sets he smeared pure cultures of *Bacillus amylovorus*, while the third set was used as a control. The cuttings, of an unnamed variety, were in a "semidormant condition with the leaves slightly started into growth." They "somewhat reluctantly and somewhat feebly it is true, but nevertheless definitely, developed * * * blight" on the inoculated surfaces. Waite notes that before the rose cuttings developed very well marked cases of blight molds began to creep over the surfaces, putting an end to the experiment. In view of these facts it seemed desirable to ascertain the action of the fire-blight organism on roses without placing the inoculated parts under such "forced" conditions."

Good-sized twigs bearing flower buds were cut from a Fairfax rose which was making rapid growth out of doors. These cuttings were placed with their cut ends in water and inoculated by means of needle punctures as well as by injections with a hypodermic needle. Others, serving as checks, were wounded similarly and kept on a greenhouse bench close to the inoculated ones. No effort was made to prevent the inoculum from drying out or to keep the inoculated parts in an atmosphere surcharged with water. On April 10 some

15 inoculations were made, some on the newly developed twigs, others on the pedicels of the blossoms, and the remaining ones in the young ovaries. By April 13, three days after the inoculations were made, a number showed clear signs of blight in the form of darkened, almost blackish discolorations and a withering of the attacked parts. By the fifth day all of the inoculations showed blackish areas around the inoculated points extending in a number of cases for several inches beyond the centers of infection. (See Fig. 3.) All the checks remained healthy and there were no signs of discoloration or collapsing of tissues around the wounds. The disease appeared equally severe on the twigs, pedicels, and ovaries. In the last-named the disease producer invaded almost the whole flower bud, discoloring and killing the whole of the calyx, often including the tips of the lobes and a considerable part of the petals. Some of these blighted buds were placed in an ice chest and they developed typical oozing of the germs within 48 hours (fig. 4), the drops of ooze studding a large part of the infected areas. From one of the diseased buds the pathogene was recovered in pure culture and its virulence established by injecting it into healthy pear shoots and producing typical blight.

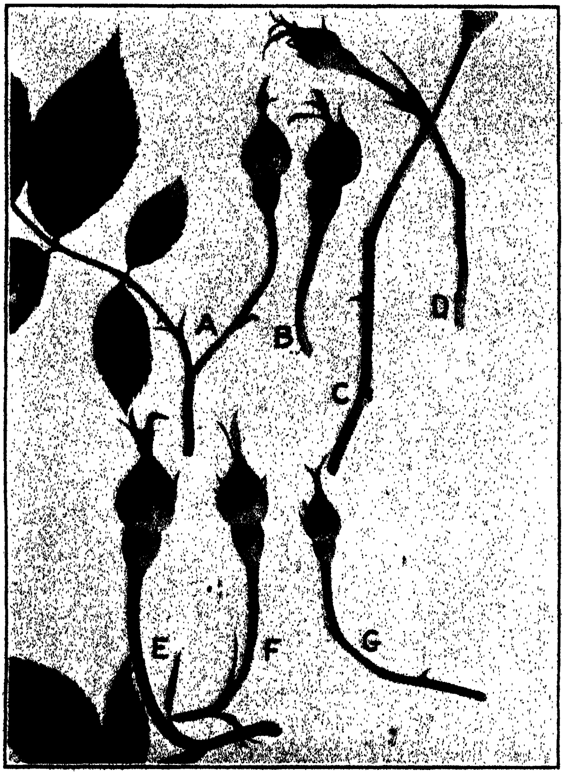

FIG. 3.—Artificial infections on twigs and flower buds of Fairfax rose: A, B, C, and D represent successful artificial infections; E, F, and G served as controls. Note the blackish discolored tissues of C and D, the discolored floral bud B, and the infected pedicel, A. Photographed four days after inoculating

As only a limited amount of growth was obtained in these cut twigs subsequent to their excision, the blight would not be expected to travel for any considerable distance beyond the inoculation centers, and in no case did it extend more than about 4 inches. But, even assuming that the blighting would have been more extensive had the inoculations been made on unsevered, vigorously growing shoots, there were no indications in these artificial infections that the disease is capable of producing any severe infections on the plant as a whole. Nevertheless in this host, as in the flowering quince, the ease with which infections may be produced suggest the possibility of natural infections occurring readily and being overlooked because of their insignificant size and the slight damage that they occasion.

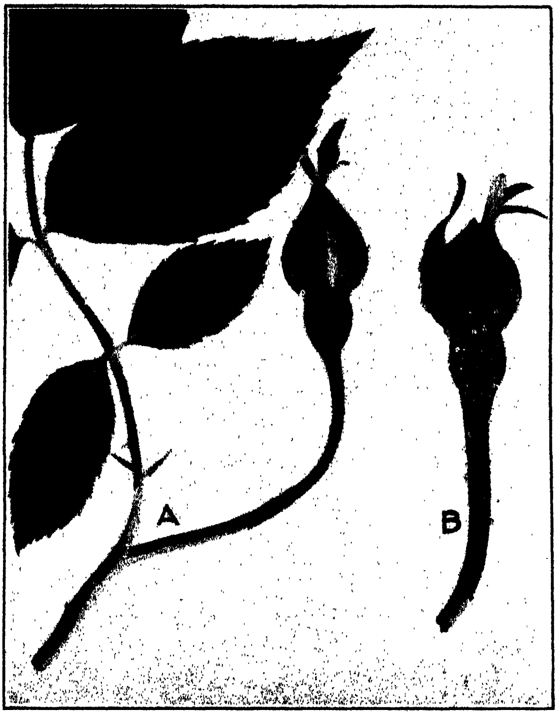

FIG. 4.—A and B, Artificial infections on rose showing large quantities of bacterial ooze, which appeared as globular, whitish drops on the calyxes, receptacles, and pedicels. Photographed six days after the inoculations were made

ARTIFICIAL INFECTIONS ON SPIREA

Having successfully produced infections on two different ornamental plants belonging to the rose family, it appeared worth while to attempt infections on the very common Spirea, *Spiraea vanhouttei* Zabel. As far as the writers know there are no records of the disease having been reported on this or other species of Spiraea in spite of the fact that the species noted above, as well as others, constitute some of the most frequently used ornamental plants in America. Unfortunately a severe frost injured almost all of the bloom as well as some of the tender shoots, ruining incidentally the first attempts at artificial infection that had been made out of doors. Because the blossoms were not available, efforts were then confined to vigorously growing leafy shoots. A number of twigs were severed from a plant and placed with their cut ends in water, the twigs after inoculation being kept in the greenhouse. The method of inoculation was exactly the same as that used on Japanese quince and on rose, and resulted in typical blight (fig. 5) of 12 Spirea twigs within five days after inoculation. All control twigs remained healthy. The disease on this host differs somewhat in early symptoms from those previously given. The attacked parts which in most of the twigs involved several inches rendered the tissues extremely flaccid, in addition to the customary discoloration. But the odd effect was the appearance of the leaves attached to the diseased parts of the twig, which remained greenish and otherwise healthy looking for a considerable number of days after the twigs had contracted the disease. Eventually they succumbed, becoming brownish and gradually withering and dying completely. In the first few days after infection, however, the disease only involved the twigs and the lowermost parts of the leaf petioles, giving the leaf tissues the appearance of extreme resistance to the invading organism. Whether this is a normal reaction or whether it is merely due to the abeyance of host growth, and hence a lessening in susceptibility because of the twigs being severed, remains to be determined. It should be pointed out, however, that while the writers' artificial twig infections of pears have almost always led to a subsequent infection of subtending leaves, resulting in bacterial penetration of parts of the midrib and adjoining tissues, this was not the case in the Spirea infections. The subsequent death of the leaves may be considered as a secondary effect resulting from the killing of the adjoining twig portions. From one of the diseased twigs the organism was recovered and its pathogenicity established by inoculating pear twigs.

The general appearance of the disease in Spiraea, as in flowering quince, is markedly similar to the injury caused by frost. When diseased material is placed beside material injured by frost it is next to impossible to distinguish one from the other, and considering the fact that the early growth of Spiraea frequently occurs at a time of late spring frosts, at least in this section of the country, it is quite conceivable that the disease may occur naturally on this host without being detected. Inasmuch as no oozing of bacteria was obtained in any of the artificial infections, the disease on this plant is all the more apt to be confused with frost injury.

NATURAL INFECTION ON BURBANK PLUM

A number of drupaceous plants, mostly plum and cherries, have at times been reported as susceptible to *Bacillus amylovorus*. The first definite proof of susceptibility of a species of Prunus was advanced by L. R. Jones (*16*), who in 1902 with the assistance of L. P. Sprague, cultured the pathogene from blighted Cheney plum, *P. nigra* Ait. (*P. americana* var. *nigra* Waugh), and produced typical infections on green pear fruits, a growing pear seedling, and on green plums, later recovering the organism from these artificial infections. M. B. Waite, according to Jones and to Smith (*22*,

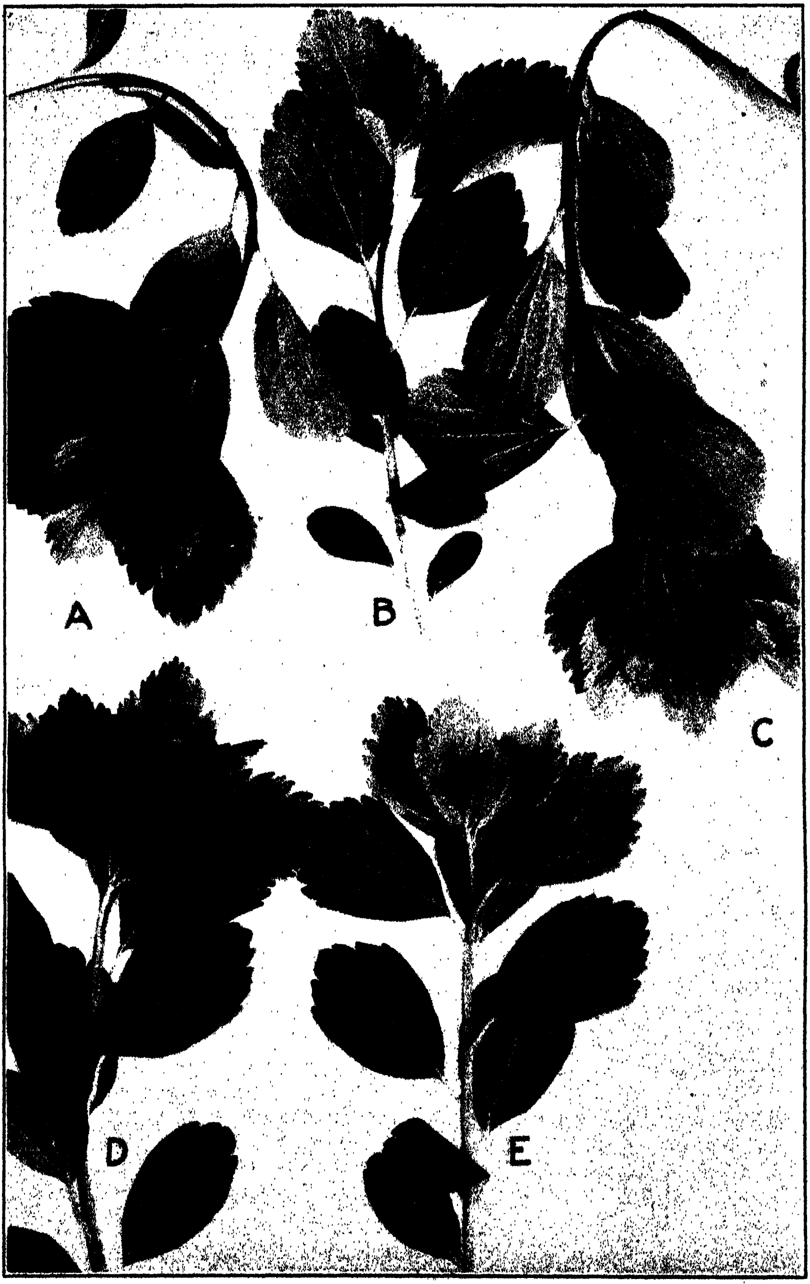

FIG. 5.—Artificial infections with *Bacillus amylovorus* on *Spiraea vanhouttei:* A, B, and C, Shoots inoculated with pure cultures by means of a hypodermic needle; D and E controls injected with sterile water. Photographed five days after inoculation

p. 359), also cultured the organism from blighted plums, but the varietal name is not given. Jones also reported that Waugh had observed the disease on hortulana plum, *P. hortulana* Bailey. In 1903 Paddock (*19*) showed that the blight on apricots was due to *B. amylovorus* and without giving proof he suggested that the blight on the apricot plum, *P. simonii* Carr, was due to the same organism. Whetzel (*27*) reported in 1909 "what appeared to be this disease killing prune trees" and Jackson (*15*) in 1915 fully substantiated this suggestion. The only other reference that has been found to plum or plumlike fruits which involved the fire-blight disease is that by Hotson (*14*) in 1916 who noted and figured blighted twigs on the yakimine, a cross between a prune and a peach, but he made no effort to obtain pure cultures and produce artificial infection.

It appears from the literature just cited that no one has noted the disease on any of the varieties of Japanese plum, *Prunus salicina* Lindl. (*P. triflora* Roxb. ex Bailey). It is therefore of interest to note that the Burbank plum, one of the varieties of the Japanese plum, was found by the senior writer to be blighted in May, 1928, in the region around Fayetteville, Ark. The disease was attacking twigs and leaf clusters of a single tree that was growing close to some badly blighted apple and pear trees. The damage was very slight, and in this respect is similar to that noted by other investigators of this disease on stone fruits in general. The organism was found within the attacked tissues and pure cultures were obtained. When grown on various culture media it appeared very similar to other strains obtained from apple and pear and when it was inoculated on vigorously growing pear shoots in the greenhouse it produced typical blight. From one of these blighted pear shoots the pathogene was recovered and from its cultural reactions was readily identified as typical *Bacillus amylovorus*. While the observations noted above indicate quite definitely that the disease is of minor importance on Burbank plum, it is necessary to bear in mind that there still is the possibility that the organism may be carried over winter in such hosts and serve as inoculating centers for susceptible host plants.

THE RANGE OF HOSTS OF BACILLUS AMYLOVORUS

The fact that new hosts have been added from time to time and that some of them, including very recent additions, have been published in journals not primarily devoted to research suggests the desirability of bringing them together in a list for ready consultation. It should be noted that a relatively large number of plants which have been reported by various individuals as subject to this disease are excluded from the list because the evidence is lacking. Among others, peach, almond, red raspberry, and blackberry, listed by Hewitt (*12*), are not included. No review of the literature concerning hosts will here be attempted in view of the excellent summary presented by Snow (*23*) in 1922. It may be worth while, however, to give the reasons for presenting some of the data in the list. There are very good reasons for believing that the disease has been recognized on some pomaceous plants since the latter part of the eighteenth century, as Arthur (*1*) first pointed out; but considering the fact that the true cause of the disease remained unknown until Burrill's time in 1878 (*7*), it becomes difficult to assign authorities for some of the hosts. There can be little doubt that if one adheres rigidly to Koch's rules of proof (*17*) even Burrill's name would be excluded, since it is very questionable whether he worked with pure cultures. Nevertheless a reasonable consideration of his pioneer work on this disease must include his name as the authority for some of the hosts.

LIST OF PLANTS [a] SUSCEPTIBLE TO BACILLUS AMYLOVORUS

Scientific name	Common name	Investigators	Date
Amelanchier canadensis	Service berry	Arthur (*1*)	1885
Chaenomeles lagenaria (*Cydonia japonica*).	Flowering quince	Rosen and Groves	1928
Crataegus crusgalli	Cockspur thorn	Reed (*20*)	1914
Crataegus oxycantha	English hawthorn	Arthur (*1*)	1885
Crataegus oxycantha var. *splendens*	Double scarlet variety of English hawthorn.	Edwards (*9*)	1907
Crataegus pyracantha (see *Pyracantha coccinea*).			
Cydonia oblonga (*C. vulgaris*)	Quince	Burrill (*7*)	1881
Eriobotrya japonica	Loquat	Waite (*24*)	1907
Fragaria spp	Strawberry (wild and cultivated varieties).	Munn (*18*)	1918
Heteromeles arbutifolia	Tollon (Christmas berry)	Waite (*24*)	1907
Mespilus sp. [b]	Medlar	Waters (*8*, *26*)	1921, 1922
Prunus armeniaca	Apricot	Paddock (*19*)	1903
Prunus avium	Royal Ann cherry and Bing cherry varieties.	Hotson (*13*)	1915
Prunus domestica	Prune	Whetzel, in Whetzel and Stewart (*27*), and Jackson (*15*).	1909, 1915
Prunus hortulana [b]	Hortulana plum	Waugh, in Jones (*16*).	1902
Prunus nigra (*P. americana* var. *nigra*).	Cheney plum	Jones (*16*)	1902
Prunus simonii [b]	Apricot plum	Paddock (*19*)	1903
Prunus triloba var. *plena*	Flowering almond	Snow (*23*)	1922
Pyracantha coccinea	Common fire thorn or evergreen thorn.	Arthur (*1*)	1885
Pyrus amygdaliformis	Chinese wild pear	Reimer (*21*)	1925
Pyrus baccata (or one of its hybrids)	Siberian crab (common crab)	Arthur (*2*)	1885
Pyrus balansae	Chinese wild pear	Reimer (*21*)	
Pyrus betulaefolia	do	do	1925
Pyrus bretschneideri	do	do	1925
Pyrus calleryana	Chinese pear	do	1925
Pyrus calleryana-dimorphophylla	Chinese wild pear	do	1925
Pyrus canescens	do	do	1925
Pyrus communis	Cultivated (European) pear	Burrill (7)	1881
Pyrus cordata	Chinese wild pear	Reimer (*21*)	1925
Pyrus coronaria	Wild garland crab	Arthur (*4*)	1887
Pyrus cotinifolia	Chinese wild pear	Reimer (*21*)	1925
Pyrus elaeagrifolia	do	do	1925
Pyrus fascicularis	do	do	1925
Pyrus faurieri	do	do	1925
Pyrus glabra	do	do	1925
Pyrus heterophylla	do	do	1925
Pyrus hondoensis	do	do	1925
Pyrus koehnei	do	do	1925
Pyrus longipes	do	do	1925
Purus malifolia	do	do	1925
Pyrus malus	Cultivated apple	Burrill (7)	1881
Pyrus mamorensis		Reimer (*21*)	1925
Pyrus michauxii		do	1925
Pyrus nivalis		do	1925
Pyrus ovoidea (hybrid?)		do	1925
Pyrus parviflora		do	1925
Pyrus paschia	Chinese wild pear	do	1925
Pyrus persica	do	do	1925
Pyrus phaeocarpa	do	do	1925
Pyrus salicifolia	do	do	1925
Pyrus serotina	Oriental pear	do	1925
Pyrus serrulata (hybrid?)	Chinese wild pear	do	1925
Pyrus sinaica	do	do	1925
Pyrus ussuriensis	Oriental pear	do	1925
Rosa sp	Unnamed cultivated variety and Fairfax rose.	Waite (*25*) and Rosen and Groves.	1925, 1928
Sorbus americana	American mountain ash	Burrill (7)	1881
Sorbus aucuparia var. *laciniata*	European mountain ash	Edwards (*9*)	1907
Spiraea vanhouttei	Vanhoutte spirea	Rosen and Groves	1928

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[a] The scientific names used are those given by Bailey (*5*) except for the various oriental pear species, for which Reimer (*21*) is accepted as the guide.

[b] Complete evidence still wanting.

SUMMARY

A knowledge of the host range of *Bacillus amylovorus* is not only important from the standpoint of possible control measures but it also makes possible the study of parasitism in an exceptional bacterial species, one which, in spite of its wide host range, is able to penetrate into at least one kind of natural opening in numerous though diverse species and genera of rosaceous plants.

Three new hosts are here presented and confirmation afforded of another. They are, respectively, the common Japanese or flowering quince, the Vanhoutte spirea, the Burbank plum, and a cultivated rose of the Fairfax variety.

The Japanese quince, *Chaenomeles lagenaria*, was found to be susceptible in artificial infection experiments on blossoms and twigs, the pathogene being able to infect the blossoms very readily through the nectaries. No natural infections on this host have as yet been found and attention is called to the fact that the gross symptoms of the disease are very much like frost injury.

Artificial infections are readily accomplished on young twigs of Vanhoutte spirea, *Spiraea vanhouttei*, with symptoms comparable to frost injury on this host.

Natural infections have been found on the Burbank plum, *Prunus salicina*, from which the parasite was obtained in pure culture and was shown to be infectious on pear shoots.

Blossoms and young twigs of Fairfax rose are here described as being very susceptible to *Bacillus amylovorus* in artificial infections.

A list of all the known species of host plants is presented.

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TIME - TEMPERATURE RELATIONS IN DIFFERENT TYPES OF PEACH-ROT INFECTION[1]

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INTRODUCTION

Car-lot shipments of peaches frequently show considerable spoilage upon arrival at their destination, and this spoilage is usually in the form of Monilia and Rhizopus rots. The behavior of these rots at various constant temperatures was described in an earlier publication.[2] Data were also reported on the effect of delays in cooling. The present paper reports further studies on the temperature responses of Monilia and Rhizopus, includes experimental data on their behavior in a gradually falling temperature such as prevails in a refrigerator car, shows the contrasts in the incubation period at different temperatures and with different types of inoculation, and attempts to equate the different growth and incubation values. It also gives shipping results from sprayed and unsprayed fruit.

EQUATION OF TEMPERATURE VALUES

METHODS

Experiments were made at various constant temperatures with different types of inoculation, and the various responses were reduced to a basis of comparison. Three different methods of inoculation were used: (1) Forcing the spores into the peaches with a needle, (2) puncturing the peaches and then dusting them with spores, and (3) dusting unpunctured peaches.

The needle inoculations were made by pushing spores to a depth of 3 to 6 mm. into the flesh of the peach with a coarse needle. The dusting inoculations were made by placing the fruit in a large paper bag along with rotten but firm peaches carrying a good covering of spores, and rolling the two lots gently back and forth from one end of the bag to the other. In the Rhizopus dusting experiments, fruiting Petri-plate cultures were sometimes substituted for the rotten peaches as inoculation material.

As mentioned above, a part of the dusted peaches had been previously punctured. Except where otherwise stated, 10 punctures were made on each peach with No. 18 wire (paper-clip wire). As a matter of convenience wires were mounted in a large cork at distances of 12 to 15 mm. apart, and this group of wires was forced first into one side of the peach and then into the other.

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[1] Received for publication Aug. 17, 1928; issued December, 1928.

[2] BROOKS, C., and COOLEY, J. S. TEMPERATURE RELATIONS OF STONE FRUIT FUNGI. Jour. Agr. Research 22: 451-465, illus. 1921.

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The peaches used in the experiments were selected with great care so that those of the different lots would be alike in maturity, in size and color, and in freedom from evident bruises and blemishes. Eight to ten peaches were used under each condition tested, and most of the experiments were repeated fifteen to twenty times. Before the inoculations were made [3] the peaches were cooled to approximately the temperature at which they were later to be placed.

The fungi used in the inoculations were obtained from active peach rots. They were apparently *Sclerotinia fructicola* (Wint.) Rehm and *Rhizopus nigricans* Ehr. and are referred to in the present paper as Monilia and Rhizopus.

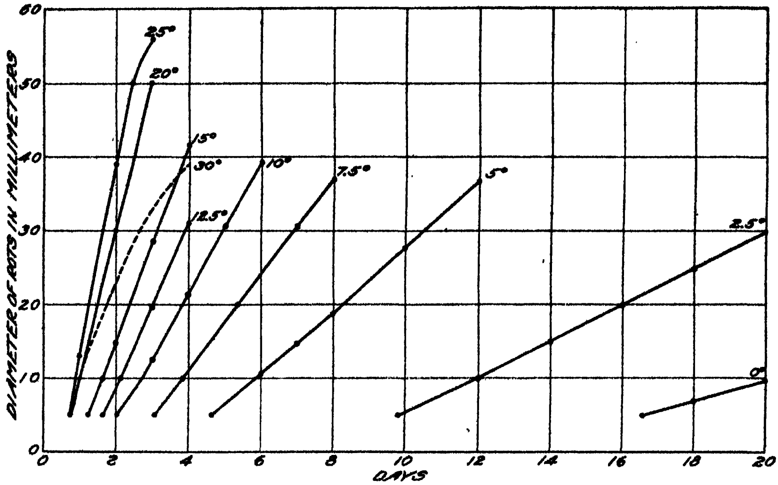

FIG. 1.—Growth curves of Monilia needle-inoculation rots on peaches at various constant temperatures; the average of results from 20 series of experiments, 1 with Carman, 4 with Hiley, 8 with Belle, and 7 with Elberta. There was little contrast between the different varieties, but the results from the individual experiments sometimes varied as much as 2 per cent from the average in the rate of growth and 10 per cent from the average in the period of incubation. In the growth at 30° C. and in the incubation period at 0° and 2.5°, the variation was sometimes even greater than this. The results reported are based on peaches showing 10 to 16 pounds pressure with a standard pressure tester, but similar results were obtained at the higher temperatures and only slightly accelerated development at the lower temperatures with peaches showing 5 to 10 pounds pressure

MONILIA NEEDLE INOCULATIONS

The results with the Monilia needle inoculations at constant temperatures are shown in Figure 1. It will be seen that considerable time was required for the rots to become established, especially at the lower temperatures, but that after this incubation period they increased in diameter in a fairly uniform manner, requiring approximately the same number of hours to enlarge from a 10 mm. to a 20 mm. diameter as to enlarge from 20 mm. to 30 mm. Usually the same rate of growth continued upward to a diameter of 40 mm. or more and downward to a diameter of 5 mm. At 30° C., however, there was a continual slowing up of the growth rate after the rot had attained a diameter of 15 mm. At some of the lower temperatures the rate of increase in diameter was not always as rapid between 5 mm.

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[3] In an earlier report the inoculations were made on warm peaches. BROOKS, C., and COOLEY, J. S. Op. cit.

and 10 mm. as in the later stages of growth, but this difference has largely disappeared in the averages, as shown in Figure 1.

The rate of increase in diameter at the different temperatures is graphically shown in the smoothed curve (*a*) of Figure 2. This curve is based on the growth values shown in Figure 1 and has been found to hold both for rots started at the given temperatures and for those started under room or field conditions and then transferred to these temperatures.

It is obvious that with a uniform increase in diameter at a particular temperature, as described above, there was a progressive increase in the volume of work accomplished per hour as the rot enlarged, the actual quantity of tissue broken down in one hour during the 20 to 30 mm. stage being much greater than that broken down in one hour during the 10 to 20 mm. stage. The rots at the higher temperatures, therefore, are soon in a later and a more enlarged period of growth than those at the lower temperatures, making any temperature comparison invalid if based on volume of rot or weight of rotten tissue or on actual work accomplished in a specified hour, at a considerable period after inoculation. Comparisons based on the rate of increase in diameter, however, are not subject to this criticism, since, as pointed out above, the rate of increase is usually practically the same over the larger part of the period of growth.

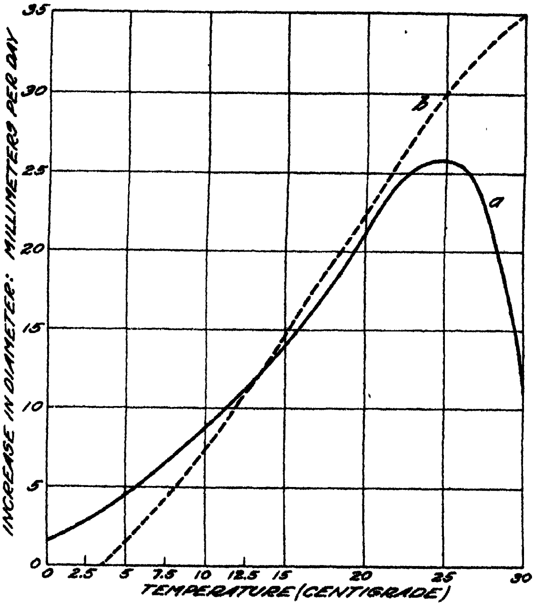

FIG. 2.—Temperature curves based on the hourly increase in the diameter of the rots as shown in Figures 1 and 9. Curve *a*, Monilia; *b*, Rhizopus

Probably the best basis for any temperature comparison is that of the number of hours required to complete a particular stage of development, especially in any study involving the incubation period. It is interesting to note that at temperatures from 15° to 25° C. approximately the same number of hours were required for the Monilia rots to get started and grow to a diameter of 10 mm. as for them to enlarge from a diameter of 10 mm. to one of 30 mm.; whereas at the lower temperatures more time was required for the first of these periods of development than for the second and at 30° much more time for the second than for the first. (See Table 1 and fig. 1.)

TABLE 1.—*Rate of development of Monilia rots at different stages and temperatures, based on the growth curves of Figure 1*

Growth period	Approximate number of hours required at stated temperatures (° C.)									
	30°	25°	20°	15°	12.5°	10°	7.5°	5°	2.5°	0°
From time of inoculation up to a diameter of 10 mm	23	21	25	39	51	65	91	142	288	480
From a diameter of 10 mm. to one of 30 mm	39	19	24	36	44	54	73	110	195	------

In developing any temperature equation for Monilia it is evident that the incubation period should be separated from the growth period and that the line of separation should be made at the earliest possible stage. The curves of Figure 1 would seem to justify making this dividing line at the point where the rots have attained a diameter of 5 mm. (0.2 inch). Taking this as the dividing line, it is seen that at 30° C. the incubation period was about 19 hours; at 25°, 17.5 hours; at 20°, 19.5 hours; at 15°, 31 hours; at 12.5°, 40 hours; at 10°, 50 hours; at 7.5°, 75 hours; at 5°, 112 hours; at 2.5°, 235 hours; and at 0°, 400 hours. These values are shown in curve *a* of Figure 3.

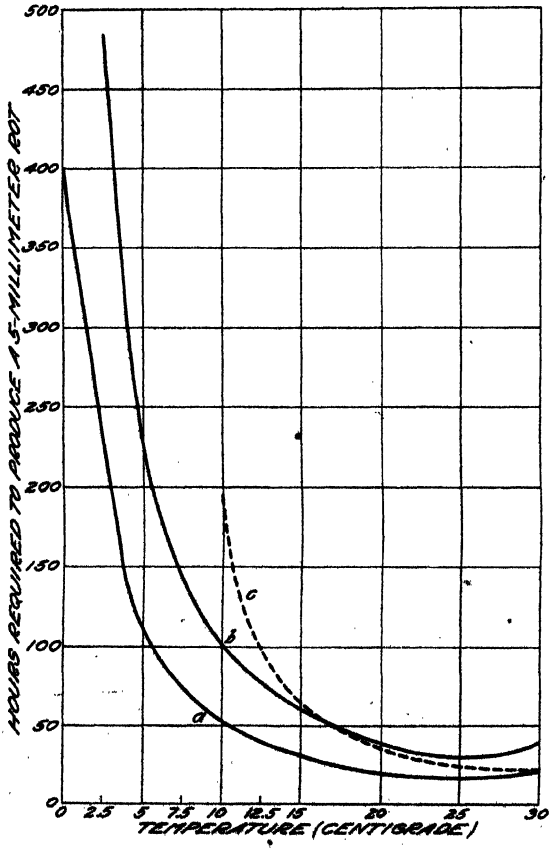

FIG. 3.—Temperature curves based on the average number of hours required to produce a 5-mm. rot as shown in Figures 1, 5, and 7. Curve *a*, Monilia needle inoculations; *b*, Monilia dusting inoculations on punctured peaches; *c*, Rhizopus needle inoculations. Curve *b* can also be regarded as showing the approximate number of hours required to bring the percentage of infection up to 20

Since these records in hours are a measure of the time required to do a certain volume of work, the reciprocals of these hour values give the relative work accomplished at the different temperatures in a given time. These reciprocal values are the basis for curve *a* of Figure 4. It will be seen from this curve that at 5° C. about four times as much work was accomplished as at 0°, about three times as much at 7.5° as at 2.5°, about 120 per cent more at 10° than at 5°, about 90 per cent more at 12.5° than at 7.5°, about 60 per cent more at 15° than at 10°, about 60 per cent more at 20° than at 15°, about 12 per cent more at 25° than at 20°, and about 10 per cent less at 30° than at 25°.

The above temperature contrasts for the incubation period should be compared with the temperature contrasts for the growth period as shown in curve *a*, Figure 2. A study of this curve shows that the growth at 5° C. was about three times as fast as at 0°, the growth at 7.5° about two and six-tenths times that at 2.5°, the growth at 10° about twice that at 5°, the growth at 12.5° about 75 per cent greater than that at 7.5°, the growth at 15° about 55 per cent greater than at 10°, the growth at 20° about 50 per cent greater than at 15°, the growth at 25° about 25 per cent greater than at 20°, and the growth at 30° less than half that at 25°. It will be seen that at the lower temperatures a 5° change had greater significance in the incubation stage of the fungus than in the growth stage and that with both incubation and growth the effect of a 5° change was far greater at the lower temperatures than at the higher ones.

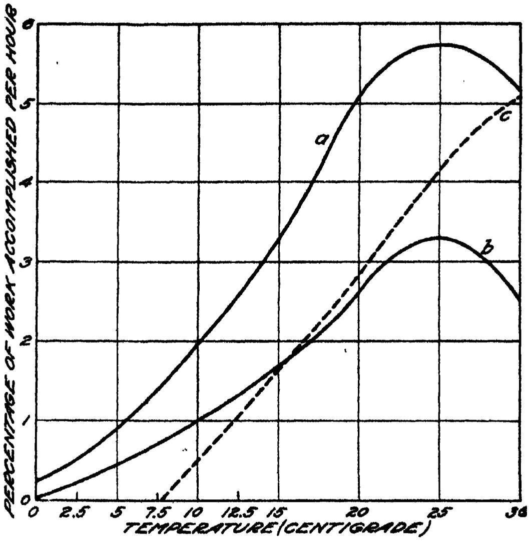

Fig. 4.—Temperature curves for the incubation period, showing the percentage of work accomplished per hour based on the reciprocals of the values shown in Figure 3. Curve *a*, Monilia needle inoculations; *b*, Monilia dusting inoculations; *c*, Rhizopus needle inoculations

MONILIA DUSTING INOCULATIONS

With needle inoculations all of the rots at a particular temperature usually started at practically the same time, but with the peaches that were punctured and dusted some of the rots were often twice as long as others in starting.

The time at which the rots appeared on the punctured and dusted peaches at the various temperatures is shown in Figure 5. At 25° C. about 10 per cent of the punctures had rots by the end of the first day and 76 per cent by the end of the second day; at 20°, 8 per cent the first day, 42 per cent the second day, and 96 per cent the third day; at 30°, 36 per cent the second day and 54 per cent the third day; at 15°, 19 per cent the second day and 78 per cent the fourth day; at 10°, 12 per cent the fourth day and 72 per cent the sixth day; at 5°, about 3 per cent the sixth day, 16 per cent the eighth day, and 29 per cent the tenth day; and at 2.5°, about 6 per cent the thirteenth day and 30 per cent the nineteenth day. It will be seen that the lower the temperature the greater was the period of time over which infections occurred and that there was a fairly close ratio between the spread of the infection period and the time required to produce the first rot. The final percentage of infection (fig. 5) was much higher at temperatures near the optimum for the fungus than at those distinctly below or above the optimum. This was largely a direct temperature response, but one other factor should be mentioned. The discontinuance of infection records was usually brought about by the absorption of the uninfected punctures in the growth of the rots already started. At the lower temperatures growth was not inhibited to the same relative degree as infection, and therefore the records may have been closed relatively earlier at these temperatures.

The growth curves for the rots resulting from the dusting inoculations on punctured peaches are shown in Figure 6. A comparison of the curves of Figures 5 and 6 with those of Figure 1 shows that the rots from the dusting inoculations were greatly delayed as compared with those from the needle inoculations. This is brought out more strikingly in the curves of Figure 3, showing the number of hours required for the diameters of the rots to reach an average of 5 mm. and those of Figure 4, showing the relative volume of work accomplished per hour during the incubation period. It will be seen that it took approximately twice as long for a rot to get started when the spores were dusted over a puncture as when they were forced into the flesh with a needle. At 25° C. it did not take quite twice as long, and at 5° and 2.5° it took more than twice as long.

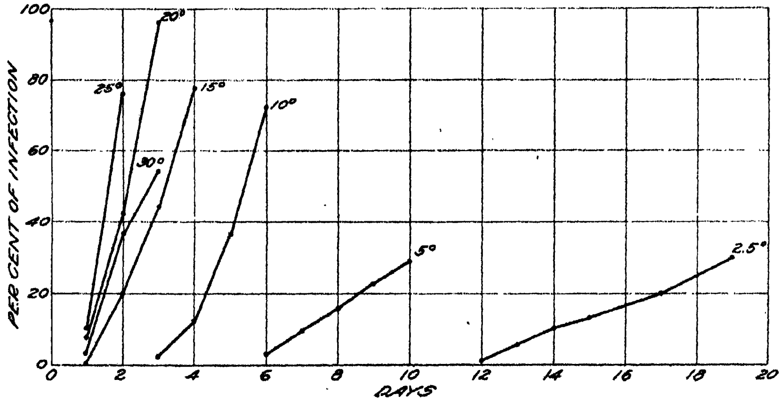

FIG. 5.—Temperature infection curves for peaches dusted with Monilia spores. Each peach had received 10 punctures previous to dusting, and the curves show the percentages of infection at these punctures after the various periods of time indicated on the base line. The results are the average of 16 series of experiments. (See legend of fig. 6)

These incubation values for the dusted peaches are based on the number of hours required for the average diameter to reach 5 mm. and are not identical with values obtained by taking an average of the number of hours required to produce a 5-mm. rot. With the latter method of computation all of the rots that eventually appeared at the punctures would be considered in the earlier as well as the later valuations, and the number of hours required for the incubation period as shown in Figure 3 would be thus increased approximately 30 per cent. This computation would make the incubation period for the punctured and dusted peaches more than two and a half times as long as that for the needle inoculations, instead of twice as long, as reported above.

Two reasons can be assigned for the earlier development of the rots from needle inoculations than from puncturing and dusting: (1) The needle inoculations carried more spores into the flesh of the peach than would be lodged around a single puncture by the dusting method, and (2) a spore located in the moist, broken tissue of the flesh has much more favorable conditions for quick germination and the rapid production of a rot than one located on the surface of the peach at or near a puncture.

A comparison of the curves of Figure 6 with those of Figure 1 would indicate that the growth rate of the rots resulting from dust inoculations was slower than that of the rots from needle inoculations; but such was not the case. The rots when once established grew at practically the same rate, regardless of the method of inoculation; and the relatively slow growth rate indicated for the early stages of the dust inoculations is due to the fact that the continual appearance of new rots prevented the increase in the average diameter from being a true record of the actual rate of growth.

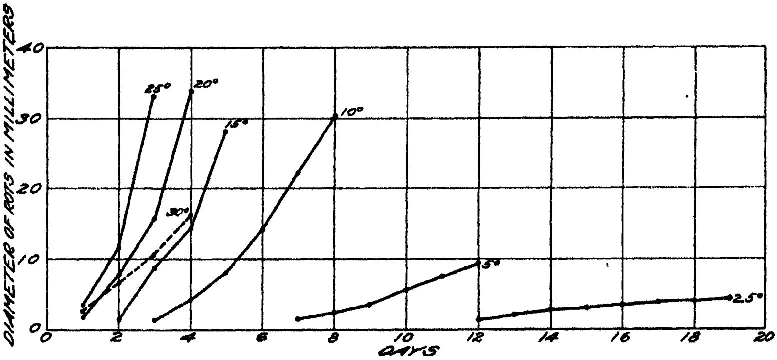

FIG. 6.—Growth curves of rots produced at various constant temperatures by dusting punctured peaches with Monilia spores. The results are the average of 16 series of experiments; 4 with Hiley, 7 with Belle, and 5 with Elberta. There was little contrast between the different varieties, but the results of the individual experiments sometimes varied as much as 50 per cent from the average, and at 5° C. and below the variation was greater than this. These variations, however, were largely between one series of experiments and another with the temperature ratios of the different series remaining fairly uniform. The results as reported are based on peaches showing 10 to 16 pounds pressure, but rots have developed with only slightly more freedom and rapidity on peaches showing 5 to 10 pounds pressure

As previously mentioned, apparently sound peaches as well as punctured ones were included in the dusting experiments. A record was also kept of the rots developing on the punctured peaches at points other than the punctures and of those developing on check peaches, which were neither punctured nor dusted. The results of these experiments have been extremely variable and very difficult to interpret. In most cases practically no rots developed from dusting unpunctured peaches with Monilia spores, but in some instances there were one-fourth to one-half as many rots on the unpunctured peaches as on the punctured ones. These rots were usually considerably delayed as compared with those occurring at the puctures.

It is possible that the extreme variation in these dusting experiments was due in part to a difference in the condition of the rotten peaches used as inoculation material, as it was observed that any juice or small particles of flesh from the rotten peaches might be an aid to infection when lodged upon the sound ones. Great care was taken, however, to avoid this by selecting inoculating material that was in a firm condition. It is also possible that rolling the peaches back and forth in the bags sometimes resulted in small punctures or abrasions that were of material assistance in the starting of the rots. The writers do not question the ability of germinating Monilia spores to gain entrance to the peach without the aid of punctures, but the variable and often negative data that they have obtained on apparently sound peaches, as compared with the almost certain infection in the presence of punctures, convince them that Monilia spores are not as great a source of infection during the usual periods in transit as is commonly supposed, except when aided by bruises, punctures, or similar favoring conditions.

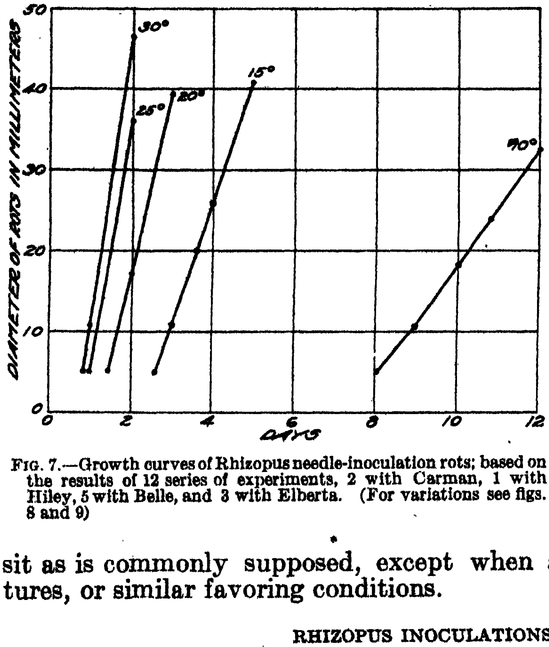

FIG. 7.—Growth curves of Rhizopus needle-inoculation rots; based on the results of 12 series of experiments, 2 with Carman, 1 with Hiley, 5 with Belle, and 3 with Elberta. (For variations see figs. 8 and 9)

RHIZOPUS INOCULATIONS

The results with Rhizopus were less uniform than those with Monilia. Even at the higher temperatures, the maturity of the fruit, the condition of the fungus, and other factors seemed to have an important modifying influence, and at 15° C. and below the results were quite variable, especially as to the period of incubation and percentage of infection.

The data for the Rhizopus needle inoculations on fruit that had been previously cooled to approximately the temperature at which it was to be stored[4] are shown in Figures 7 and 8, and the rate of growth for rots that had started before being placed at the various temperatures is shown in Figure 9. The rate of increase in diameter at the various temperatures is shown in the smoothed curve *b* of Figure 2 and the incubation period in curve *c* of Figure 4.

It will be seen that the early development of Rhizopus was much slower than that of Monilia, especially at the lower temperatures,

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[4] In an earlier report the inoculations were made on warm peaches. BROOKS, C., and COOLEY, J. S. Op. cit.

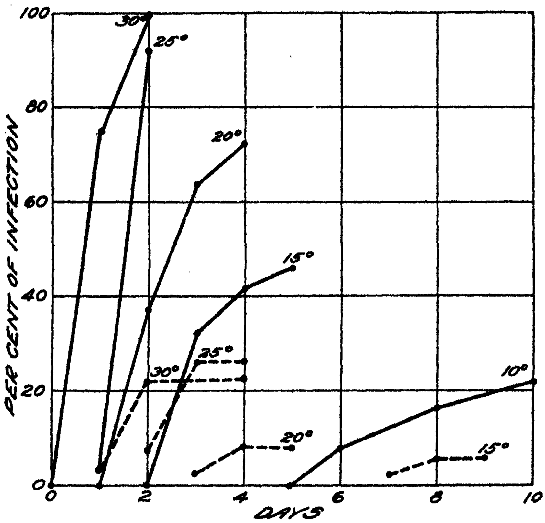

FIG. 8.—Temperature curves showing the percentage of infection with Rhizopus inoculations; solid lines, needle inoculations; broken lines, dusting inoculations on punctured peaches. With the needle inoculations there were variations from the average as great as 25 per cent at 30°, 25°, and 20° C. and as great as 100 per cent at 15° and 10°; and with the dusting inoculations there were variations from the average as great as 50 per cent at 30° and 25° and as great as 100 per cent at 20° and 15°. The variations were apparently due in part to differences in the maturity of the peaches. The needle-inoculation results are based on 12 series of experiments. (Fig. 7.) The dusting results are the average of 5 series of experiments, 1 with Hiley and 4 with Belle

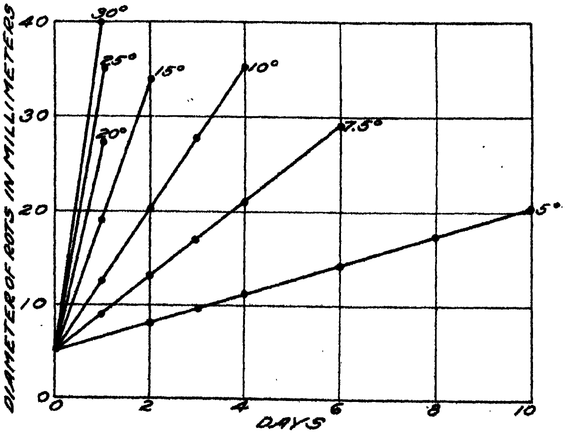

FIG. 9.—Rate of growth at different temperatures for Rhizopus rots that were already started, based on the results of 16 series of experiments at the higher temperatures and 5 series at 7.5° and 5° C. Some of the individual experiments varied from the average as much as 15 per cent. A part of this variation was apparently due to differences in the maturity of the fruit

but that when once established the rots enlarged more rapidly than those of Monilia at all of the higher temperatures, especially at 25° C. and above. Rhizopus was much more responsive to temperature changes than Monilia. It made a better development at 30° than at any other temperature tested, whereas this was above the optimum for Monilia. It was unable to start rots on peaches previously cooled to 7.5° and did not always produce them at 10°, but rots that had made a start at warmer temperatures continued to enlarge at 7.5° and even at 5°, but not at 2.5°. (Fig. 9.)

With Monilia needle inoculations, rots developed at practically 100 per cent of the punctures, and with Monilia dusting inoculations there was a very high percentage of infection at 10° C. and warmer and a gradual increase with time in the number of rots at 5° and 2.5°. (Fig. 5.) With Rhizopus, the infection from needle inoculations was not universal even at 25° and 30°, and at all lower temperatures it was very much poorer than with the Monilia dusting inoculations. (Figs. 5 and 8.) At 10° Rhizopus gave an average of only 22 per cent of infection 10 days after needle inoculation.

The infection from the Rhizopus dusting inoculations on punctured peaches was still poorer, never averaging more than 26 per cent even at the most favorable temperatures and being entirely lacking at 10° and colder. (Fig. 8.)

When once established, the Rhizopus rots enlarged at practically the same rate, regardless of the method of inoculation.

With Rhizopus, as already reported for Monilia, apparently sound peaches as well as the punctured ones were included in the dusting experiments. Rots occasionally developed on these dusted peaches, but not much more frequently than on the peaches that were not dusted. Apparently, germinating Rhizopus spores seldom if ever penetrate the sound skin of market-ripe peaches, and it is the opinion of the writers that the unbroken skin of the peach furnishes a complete protection against Rhizopus, except in cases where the sound fruit is in close contact with that which is already decayed.

DELAYS AT CONSTANT TEMPERATURES

In addition to the experiments in which the inoculated fruit was placed immediately at the temperature at which it was to be held, other tests were made in which it was delayed at a higher temperature before being placed at a lower one. The results are shown in Figures 10 to 17, inclusive.

APPLICATION OF EQUATED TEMPERATURE VALUES

So far as possible the results of the delays were interpreted in the light of the temperature studies previously reported and the actual growth compared with hypothetical growth curves developed from the temperature values of Figures 2 and 4.

As an illustration of the method of developing the hypothetical curves, the line c_1 of Figure 10 may be taken. After inoculation the peaches were held at 20° C. for 12 hours before storage at 7.5°. According to the values of Figure 4, 12 hours at 20° would allow Monilia to accomplish about 61 per cent of the work necessary to the production of a 5-mm. rot, leaving 39 per cent to be carried out at 7.5°, and according to the 7.5° values of the same figure 30 hours

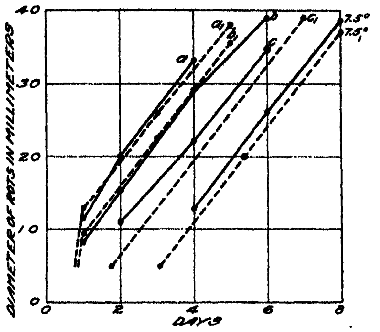

FIG. 10.—Effect of delay in cooling peaches that were needle inoculated with Monilia; the average of the results from 3 series of experiments, 1 each on Hiley, Belle, and Elberta. The solid lines give the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, at 7.5° C. after 24 hours at 25°; *b* and b_1, at 7.5° after 24 hours at 20°; *c* and c_1, at 7.5° after 12 hours at 20°; also results at a constant temperature of 7.5°

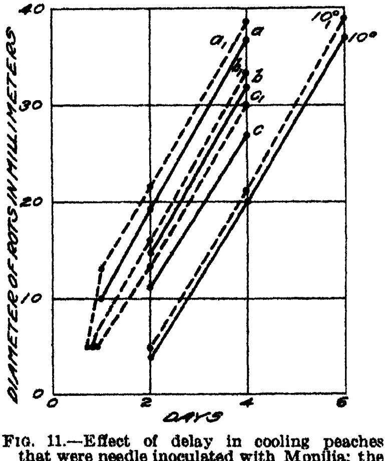

FIG. 11.—Effect of delay in cooling peaches that were needle inoculated with Monilia; the average of the results from 4 series of experiments, 1 each on Carman and Belle and 2 on Elberta. The solid lines give the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, at 10° C. after 24 hours at 25°; *b* and b_1, at 10° after 22 hours at 20°; *c* and c_1, at 10° after 18 hours at 30°; also results at a constant temperature of 10°

would be required to complete the work at that temperature, making a total of 42 hours for the incubation period. After reaching the 5-mm. stage the rots would be expected to develop according to the growth values for 7.5°, as shown in Figure 2.

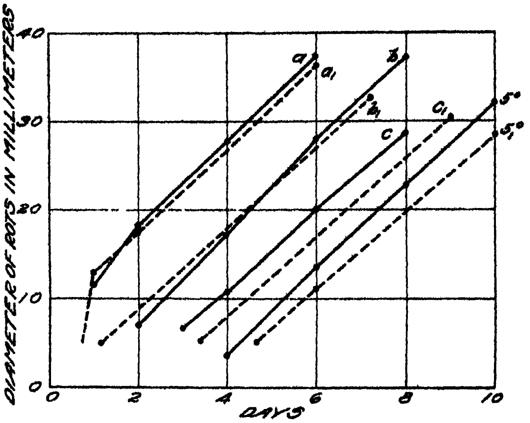

FIG. 12.—Effect of delay in cooling peaches that were needle inoculated with Monilia; the average of the results from 3 series of experiments, 1 on Hiley and 2 on Elberta. The solid lines give the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, at 5° C. after 24 hours at 25°; *b* and b_1, at 5° after 18 hours at 30°; *c* and c_1, at 5° after 12 hours at 15°; also results at a constant temperature of 5°

A comparison of the hypothetical curves for the Monilia needle inoculations with the actual growth curves (figs. 10 to 12, inclusive) shows a fairly close agreement in most cases in both the incubation period and the rate of growth. In both the delayed and immediate-storage experiments at 10° C. the actual growth was behind the hypothetical; whereas at 7.5° and 5° the actual growth was usually ahead of the hypothetical, in three instances 8 to 15 hours ahead.

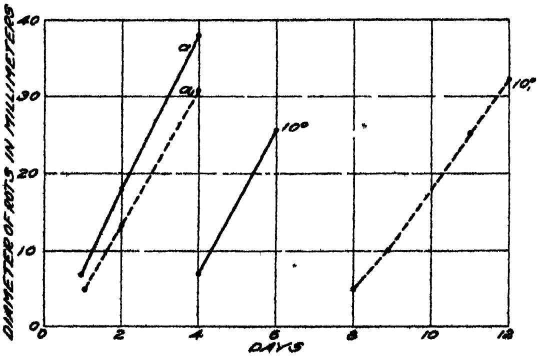

FIG. 13.—Effect of delay in cooling peaches that were needle inoculated with Rhizopus; the average of the results from 2 series of experiments, 1 each on Carman and Belle. The solid lines show the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1 at 10° C. after 18 hours at 25°. The peaches of the 10° curve were not cooled previous to inoculation

There would necessarily be a lag in temperature adjustment in moving the fruit from a higher to a lower temperature, and the fungus probably retains the stimulus of the higher temperature for a short time after removal to the lower, so it would be expected that the actual values might run somewhat ahead of the hypothetical, as developed by the methods described for Figures 2 and 4. The

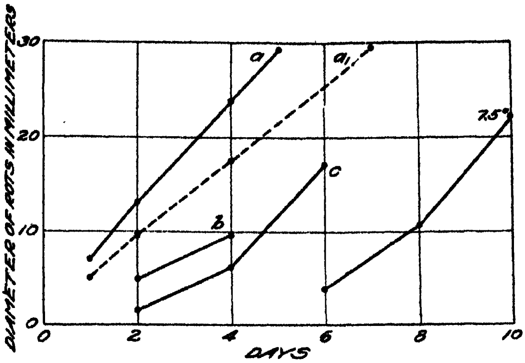

FIG. 14.—Effect of delay in cooling peaches that were needle inoculated with Rhizopus, the average of the results from three series of experiments on Belle peaches. The solid lines show the actual results, the broken line the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and *a*₁, at 7.5° C. after 24 hours at 25°; *b*, at 5° after 18 hours at 25°; *c*, at 7.5° after 12 hours at 25°. As Rhizopus does not produce a rot when placed at once at 7.5° or 5°, the values of Figures 2 and 4 do not supply the data to estimate curves for *b* and *c*. The peaches of the 7.5° curve of Figure 14 were not previously cooled

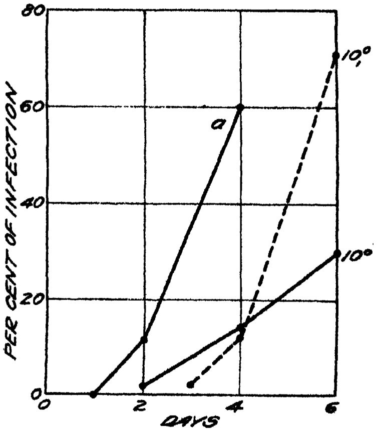

FIG. 15.—Effect of delay in cooling peaches to 10° C., as shown in the percentage of Monilia infection; the average of the results from 3 series of experiments, 1 each on Hiley, Belle, and Elberta. The peaches received 10 punctures each with No. 18 wire and were then dusted with Monilia spores. Curve *a* at 10° after 20 hours at 25°. The solid 10° line shows the results obtained in the present experiments; the broken line the average results of 16 tests as reported in Figure 5

differences between the hypothetical and the actual results, however, are little if any greater than the variation between the individual experiments at constant temperatures as described in the legend for Figure 1.

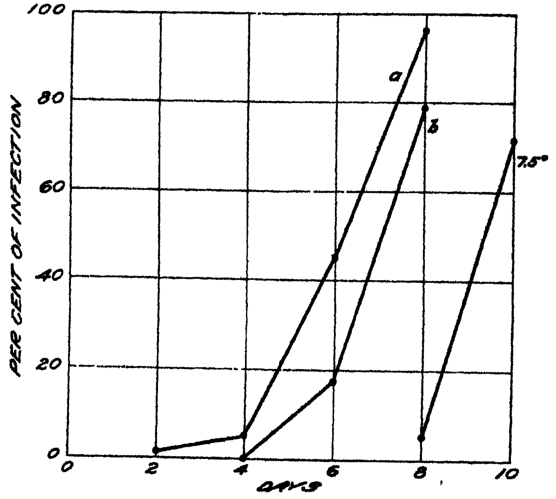

FIG. 16.—Effect of delay in cooling peaches, as shown in the percentage of Monilia infection, an experiment with Belle peaches. The peaches received 10 punctures each with No. 18 wire and were then dusted with Monilia spores. Curve *a*, at 7.5° C. after 24 hours at 20°; *b*, at 7.5° after 12 hours at 20°. The 7.5° graph shows results at that temperature held constant

In the case of the Rhizopus needle inoculations (figs. 13 and 14) the actual results were 15 to 30 hours ahead of the hypothetical.

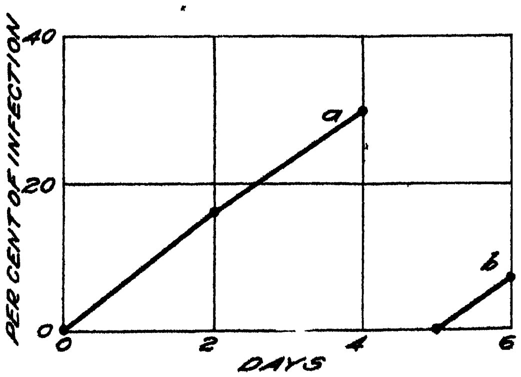

FIG. 17.—Effect of delay in cooling peaches, as shown in the percentage of Rhizopus infection, the average of the result from three series of experiments on Belle peaches. The peaches received 10 punctures with No. 18 wire and were then dusted with Rhizopus spores. Curve *a*, at 7.5° after 24 hours at 25°; *b*, at 7.5° after 12 hours at 25°. Warm inoculated peaches placed immediately at 7.5° did not develop rots

With the constant-temperature curves at 7.5° and 10° C., the contrast was much greater than this, but the peaches used in these experiments were not cooled before inoculating, whereas those upon which the hypothetical values are based were cooled to approximately the storage temperature before inoculating. With the latter method no Rhizopus growth was obtained at 7.5°, and the growth at 10° was greatly delayed.

MONILIA NEEDLE INOCULATIONS

The effect of delay in cooling on Monilia needle inoculations is shown in Figures 10 to 12, inclusive. As compared with fruit stored immediately at 10° C., a delay of 24 hours at 25° put the rots about 44 hours ahead, a delay of 22 hours at 20° put them about 33 hours ahead, and a delay of 18 hours at 30° put them about 20 hours ahead.

As compared with immediate storage at 7.5° C., a delay of 24 hours at 25° gave the rots a lead of about 72 hours, a delay of 24 hours at 20° gave a lead of about 54 hours, and a delay of 12 hours at 20° gave a lead of about 34 hours.

As compared with immediate storage at 5° C., a delay of 24 hours at 25° gave the rots a lead of about 115 hours, a delay of 18 hours at 30° gave a lead of about 67 hours, and a delay of 12 hours at 15° gave a lead of about 33 hours.

MONILIA DUSTING INOCULATIONS

The effect of delay in cooling punctured peaches that were dusted with Monilia spores is shown in Figures 15 and 16. As compared with immediate storage at 10° C., a delay of 20 hours at 25° gave a lead of about 60 hours, as shown in the number of rots. As compared with immediate storage at 7.5° a delay of 24 hours at 20° gave a lead of about 88 hours, and a delay of 12 hours at 20° gave a lead of about 56 hours.

It will be noted that a given delay in cooling had much greater effect upon the dust inoculations than upon the needle inoculations as reported above, in fact about twice the effect. This is in agreement with the relative values reported in Figure 4.

RHIZOPUS INOCULATIONS

The effect of delay in cooling on Rhizopus needle inoculations is shown in Figures 13 and 14. Figure 17 shows the effect of delay in cooling dusting inoculations. A delay of 18 hours at 25° C. before storage at 10° accelerated needle inoculation rots about 76 hours. (Fig. 13.) A delay of 24 hours at 25° before storage at 7.5° caused an acceleration of about 150 hours, and a delay of 12 hours at 25° caused an acceleration of about 74 hours, as compared with warm peaches placed immediately at 7.5°. (Fig. 14.) Rhizopus rots did not develop at 7.5° when the peaches were cooled before inoculation. A delay of 18 hours at 25° before storage at 5° made it possible for the rots to appear at the 5° temperature. (Fig. 14.)

With peaches that were punctured and then dusted with Rhizopus spores, a delay of 24 hours at 25° C. before storing at 7.5° gave the rots a lead of five days over similar inoculations on peaches that were delayed but 12 hours at 25° before storing at 7.5°. (Fig. 17.)

A comparison of the results of Figure 17 with those of Figures 13 and 14 shows that delayed cooling had a very much greater effect upon Rhizopus dust inoculation than upon the needle inoculations. With both types of inoculation the effect of delayed cooling was much greater with Rhizopus than with Monilia.

DUPLICATION OF CAR-TEMPERATURE CONDITIONS

METHOD AND EQUIPMENT

The experiments previously reported were conducted in a series of six refrigerator boxes, each having a capacity of about 1 cubic yard. Each refrigerator box was supplied with a continuous and controlled stream of brine that was held at an approximately constant temperature. It was found that by setting the brine control so that the box would be cooled to a temperature such as a car of fruit might have upon arrival at destination and then placing jars of hot water in the box a gradually falling temperature curve could be obtained similar to that of refrigerator cars. The initial temperature of the box was determined by the initial temperature of the water and the rate of cooling by the quantity of water used. The peaches were held at a distance from both the brine coils and the hot water, and the air of the box was kept in circulation by means of a slow-moving fan.

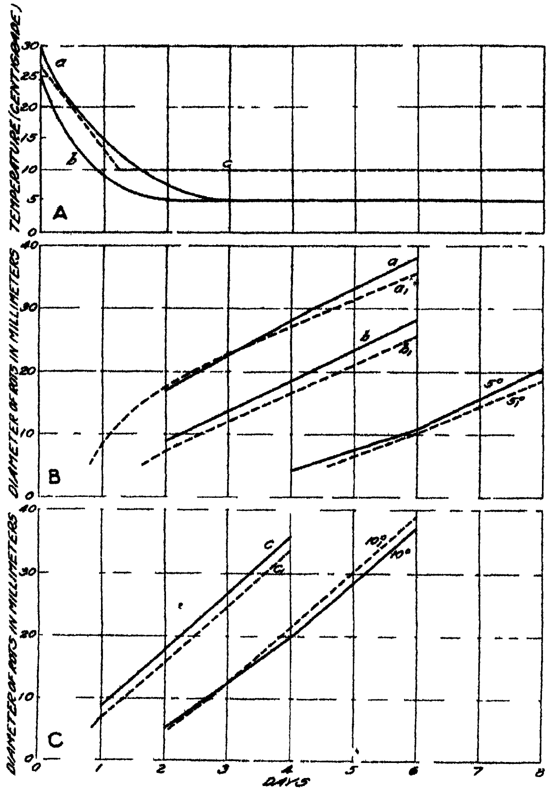

FIG 18 —A, imitation car temperature curves obtained in refrigerator boxes (see p 522) B, growth curves for Monilia (needle inoculations) on Belle peaches at the temperatures shown in the curves of A and also at a constant temperature of 5° C The solid lines give the actual results and the broken lines the estimated results based on the temperature values of Figures 2 and 4 Curves *a* and a_1, the rate of growth at the temperatures of curve A, *a*, *b* and b_1, at the temperatures of curve A, *b* C, as B, but showing the growth at the temperatures of curve A, *c*, and also at a constant temperature of 10°

Complete temperature records were obtained by means of thermographs and standard thermometers placed beside the peaches. The temperature curves thus obtained are shown at A in Figures 18 and 19.

In addition to the car-temperature conditions, constant temperatures were maintained at the same time in other boxes of the series.

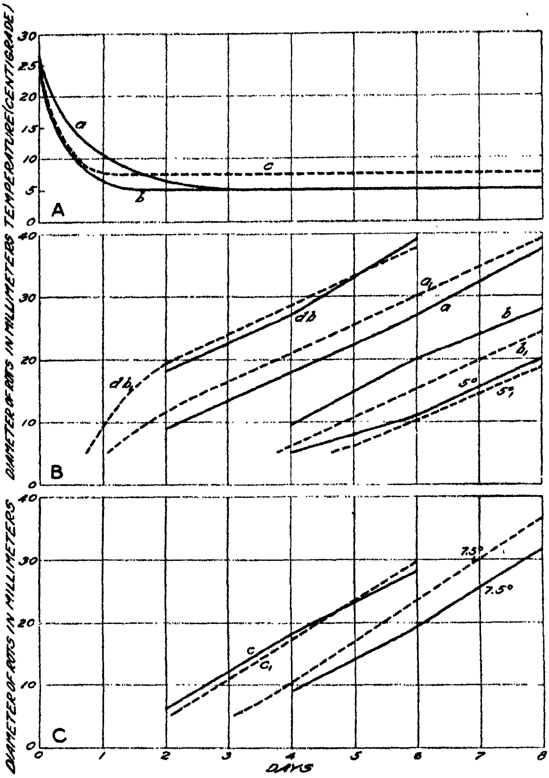

FIG. 19.—A, imitation car-temperature curves obtained in refrigerator boxes. B, growth curves for Monilia (needle inoculations) on Elberta peaches under the temperature conditions shown in curves A, *a*, and A, *b*, and also at a constant temperature of 5° C. The solid lines show the actual results and the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, rate of growth at the temperature of curve A, *a*; *b* and b_1, at the temperature of curve A, *b*; *db* and db_1, the rate of growth on peaches that were delayed at 26° for 24 hours before storing under the conditions shown in curve A, *b*. C, as B, but showing the growth of Monilia at the temperatures of curve A, *c*, and also at a constant temperature of 7.5°

APPLICATION OF EQUATED TEMPERATURE VALUES

The above conditions made it possible to hold part of a particular lot of inoculated peaches at a constant temperature while the others of the same lot were exposed to conditions similar to those that prevail in a refrigerator car. The results are shown in Figures 18, B and C, and 19, B and C. The solid lines show the actual growth and the broken lines the estimated growth based on the values of Figures 2 and 4.

The curves for the estimated growth were developed as described under "Delays at constant temperatures" (p. 516), except that instead of having two temperatures to consider there was often a different temperature for every hour. Curve a_1 of Figure 18, B, may be taken as an illustration. If the temperatures shown in the various hour periods of curve *a*, Figure 18, A, are converted into work units according to the values of Figure 4, it is found that 19 hours would be required for the Monilia rots to pass the incubation period and reach a diameter of 5 mm. They should then enlarge at a rate to be determined by converting the succeeding temperature values of curve *a*, Figure 18, A, into growth values according to the scale of Figure 2. The result is a gradually changing curve as shown in curve a_1 of Figure 18, B.

A comparison of the hypothetical Monilia curves with the actual growth curves shows practically as close agreement as was found with delays at constant temperatures. (Figs. 10 to 17, inclusive.) In five instances the actual growth was ahead of the hypothetical, in three instances behind it, and in three cases almost identical with it.

EFFECTS OF DELAYED COOLING

As compared with storing the Monilia-inoculated fruit immediately at 5° C., holding it at the temperature of curve *a*, Figure 18, A, gave the rots a lead of 130 hours; holding it at the temperature of curve *b*, Figure 18, A, or curve *a*, Figure 19, A, gave a lead of about 85 hours; holding it at the temperature of curve *b*, Figure 19, A, gave a lead of about 35 hours; and holding it at 26° for 24 hours and then at the temperature of curve *b*, Figure 19, A, gave a lead of about 135 hours. (Figs. 18, B, and 19, B.) As compared with storing inoculated fruit immediately at 7.5° C., holding it at the temperature of curve *c*, Figure 19, A, gave the rots a lead of about 38 hours. (Fig. 19, C.) As compared with storing it immediately at 10°, holding it at the temperature of curve *c*, Figure 18, A, gave the rots a lead of about 42 hours. (Fig. 18, C.)

The above accelerations in the time of the appearance of the rots resulting from the moderate delays shown in the curves of Figures 18, A, and 19, A, give evidence of the extreme importance of prompt loading and rapid cooling of harvested peaches.

PEACH ROTS IN TRANSIT

Experiments were also made in the standard refrigerator cars under commercial shipping conditions. The work was conducted in the years 1921 to 1926, inclusive, and included a record on 13 different refrigerator cars. The temperatures of Figure 20, A, are for cars shipped from Cornelia, Ga., to Chicago, Ill., but all other records are on shipments from Fort Valley, Ga., to New York City. The car-temperature curves are shown in Figures 20, A, to 30, A, inclusive.

METHODS AND EQUIPMENT

Except where otherwise stated, the cars were loaded four layers high with six-basket carriers, and the experimental peaches were placed in the middle of the stack, halfway between the bunker and the door. Half of the experimental lot was placed in the top layer and half in the bottom layer.

A thermograph was placed with each lot of peaches. It was fastened in the middle of the package, and peaches were packed around and in contact with it. It is believed that this arrangement gave the instrument practically the same temperature as the surface flesh of the fruit. The thermographs were tested before and after the shipment, and, as a further check on the instruments, the temperature of the peaches was always taken on arrival at destination. It is believed that with these various precautions a fairly accurate temperature record was obtained.

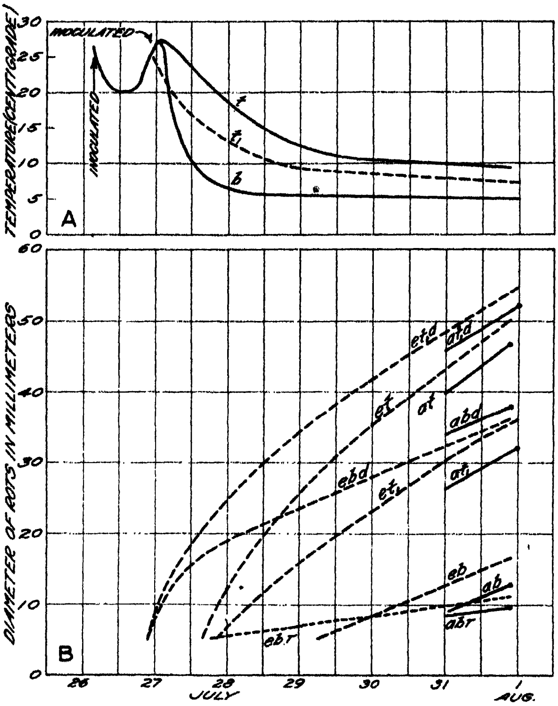

FIG. 20.—A, curves showing the temperature of peaches in two refrigerator cars that were loaded at approximately the same time, one with crates, the other with bushel baskets; also peach temperatures before loading. Curve *t*, fifth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer of the same stack; t_1, third and top layer of baskets in the sixth stack from the bunker. B, development of rots (needle inoculations) on well-matured Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves give the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with Monilia inoculations 3 hours before loading; *eb* and *ab* under the conditions of curve A, *b*, with Monilia inoculations 3 hours before loading; *ebd* and *abd* under the conditions of curve A, *b*, with Monilia inoculations 22 hours before loading; et_1 and at_1 under the conditions of curve A, t_1, with Monilia inoculations at loading; et_1d and at_1d under the conditions of curve A, t_1 with Monilia inoculations 19 hours before loading; *ebr* and *abr* under the conditions of curve A, *b*, with Rhizopus inoculations 22 hours before loading

The inoculations were made as previously described (p. 507) except that 40 or more peaches were used under each condition.

APPLICATION OF EQUATED TEMPERATURE VALUES

With peaches in transit, a record of the actual development of the rots could be obtained only at the end of the shipment. But with the temperatures known it was possible to plot hypothetical growth curves according to the values of Figures 2 and 4, as described for the imitation car temperatures (p. 524), and it was also possible to trace the probable line of growth backward, with the size of the rots at the end of the shipment as a starting point. The hypothetical growth curves thus developed for the Monilia needle inoculations and a part of those for the Rhizopus needle inoculations are shown in Figures 20 to 30, inclusive.

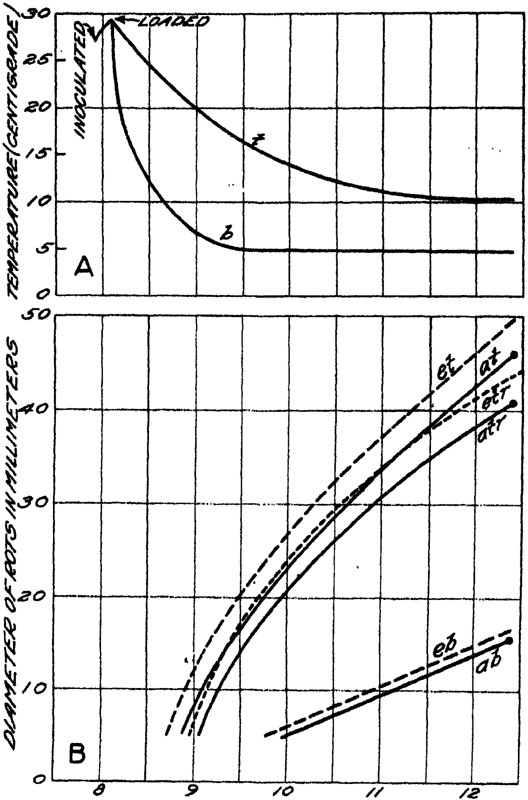

FIG. 21.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperature before loading. Curve *t*, fourth and top layer of peach crates in the sixth stack from the bunker; *b*, bottom layer two stacks from the bunker. B, development of Monilia (needle inoculations) on well-matured Elberta peaches under the temperature conditions shown in A. The average size of the rots at the destination is shown by the position of the heavy dots at the right of the figure, *at* and *atr* for the top of the car and *ab* for the bottom. The solid lines connecting with these dots attempt to trace the growth curves backward by applying the growth values of Figure 2 to curves *t* and *b* of A. Curves *et*, *etr*, and *eb* give the estimated results under the conditions shown in A, based on the incubation values of Figure 4 and the growth values of Figure 2. Curves *etr* and *atr* refer to Rhizopus, the others to Monilia. All inoculations were made approximately four hours before loading

MONILIA NEEDLE INOCULATIONS

A study of the results of the Monilia needle inoculations shows that there were 11 instances in which the actual growth of the rots was ahead of the estimated growth and 29 instances in which it was behind. In 19 cases, however, there was not more than four hours' difference in the two. If these are excluded, only 2 instances remain in which the rots were ahead of the estimated growth and 19 instances in which they were 5 to 38 hours behind it. (See "Varietal resistance," p. 529.)

As shown in Figure 4 and emphasized in later paragraphs, needle-inoculation rots make a more rapid development than other types of Monilia infection. It is therefore evident from the above results that with the temperature of the fruit known, the incubation and growth can be plotted according to the values of Figures 2 and 4 with the expectation that there will be but few, if any, instances in which the actual growth will be ahead of that which has been estimated.

MONILIA DUSTING INOCULATIONS

The results from the Monilia dusting inoculations are shown in Table 3. The rots average about one-third the size of the needle-inoculation rots exposed to the same conditions, which is approximately the ratio that holds in a comparison of Figures 1 and 6. The temperature values of Figures 4 and 6 do not form a basis for more than an approximate estimate on the results from dusting inoculations, but it can be said that in general the shipping results agree with the values shown in these figures. There are more instances in which the rots fall behind the expected results than exceed them.

TABLE 2.—*Development of Rhizopus rots on peaches in transit; needle inoculations*

Variety of peach	Temperatures as shown in—	Time of inoculation previous to loading (hours)	Condition upon arrival					
			Percentage of peaches affected		Average diameters of rots (millimeters)			
					Top of car		Bottom of car	
			Top of car	Bottom of car	Actual	Estimated [a]	Actual	Estimated [a]
Elberta	Fig. 21, A	4			41	44	0.2	0
Do	Fig. 20, A	22			Total.	Total.	9.5	11
Do	do	3			51	38	0	0
Yellow Hiley	Fig. 22, A	22			44.5	39	1.4	0
Do	do	6			18.4	21	0	0
Do	Fig. 28, A	5			.6	0	0	0
Elberta	Fig. 29, A	2			0	0	0	0
Hiley	Fig. 23, A	17	90	100	54.3	45	2.3	0-9
Do	do	2	30	0	6.3	28		
Do	Fig. 24, A	16-42	100	90	70.7	49	39.5	21
Do	do	6-32	100	70	42	37	15.0	5-10
Elberta	Fig. 25, A	14	50	20	29.2	32	3.1	0
Do	do	2	70	0	11.3	15		
Do	Fig. 26, A	16	80	50	15.4	46	3.8	11
Do	do	4	60	10	13.6	30	3.2	0

[a] Estimated on the basis of the values of Figures 2 and 4.

RHIZOPUS INOCULATIONS

The results from the Rhizopus needle inoculations are shown in Table 2 and in Figures 20 to 25. In 6 instances the growth was greater than would be expected from the values of Figures 2 and 4, in 4 instances it was less, and in 18 instances it was the same or practically the same; 10 of the 18 instances, however, were where both the actual and estimated values were zero or approximately zero.

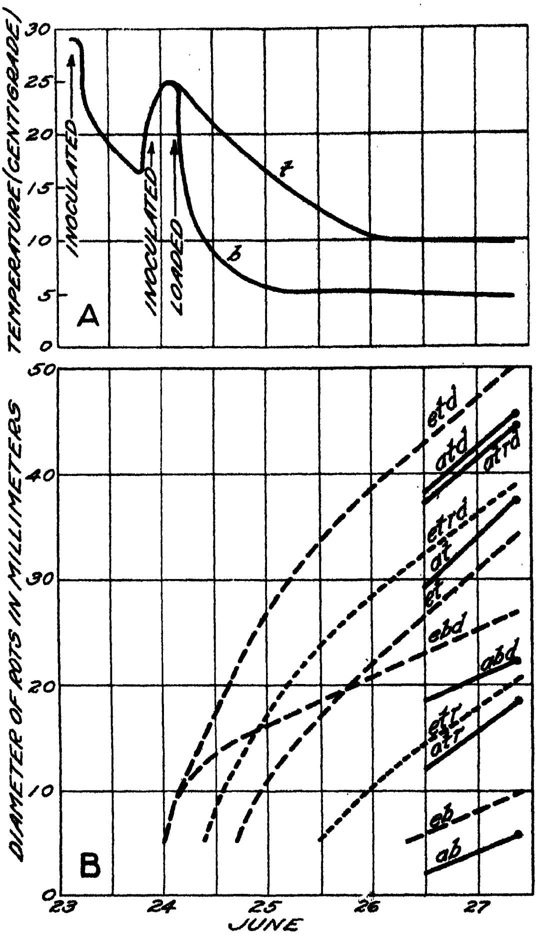

FIG. 22.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer; *b*, bottom layer in the fourth stack of crates from the bunker. B, development of rots (needle inoculations) on Yellow Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves give the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of, curve A, *t*, with Monilia inoculations 6 hours before loading; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 24 hours before loading; *eb* and *ab* under the conditions of curve A, *b*, with Monilia inoculations 6 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 24 hours before loading; *etr* and *atr* under the conditions of, curve A, *t*, with Rhizopus inoculations 6 hours before loading; *etrd* and *atrd* under the conditions of curve A, *t*, with Rhizopus inoculations 24 hours before loading

As a whole, the results indicate that the Rhizopus values of Figures 2 to 4 represent about the average of what can be expected; but the rather wide variations from this average, in some instances, give further emphasis to the erratic nature of Rhizopus rots (p. 514).

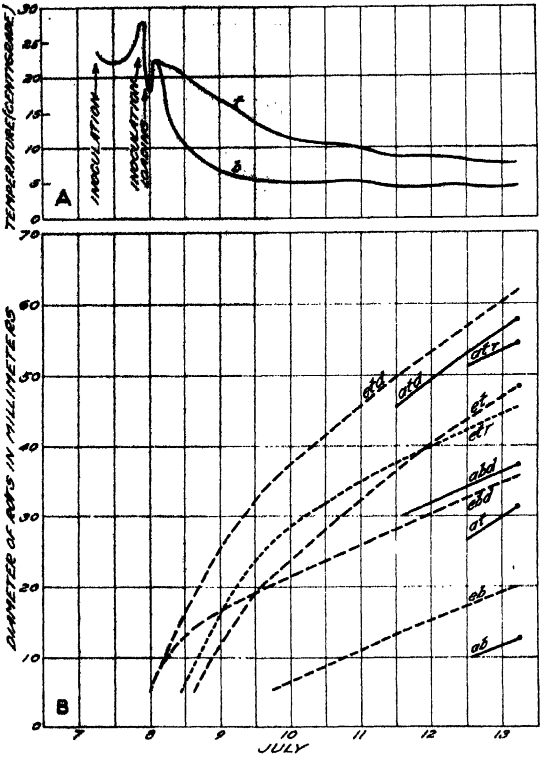

FIG. 23.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer in the same stack. B, development of rots (needle inoculations) on Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with Monilia inoculations 2 hours before loading; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 17 hours before loading; *eb* and *ab*, under the conditions of curve A, *b*, with Monilia inoculations 2 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 17 hours before loading; *etr* and *atr*, under the conditions of curve A, *t*, with Rhizopus inoculations 17 hours before loading

VARIETAL RESISTANCE

A study of the tables and figures shows that with Monilia needle inoculations on Elberta peaches the actual growth was behind the estimated in 13 instances, ahead of it in none, and approximately the same in 9. With similar inoculations on Hiley peaches the actual growth was behind the estimated in 5 instances, ahead of it in 2, and approximately the same in 11. The actual growth of Monilia on Elberta thus ran distinctly behind the estimated in 59 per cent of the tests and ahead in none. While on Hiley it ran definitely behind in only 28 per cent and ran ahead in 11 per cent.

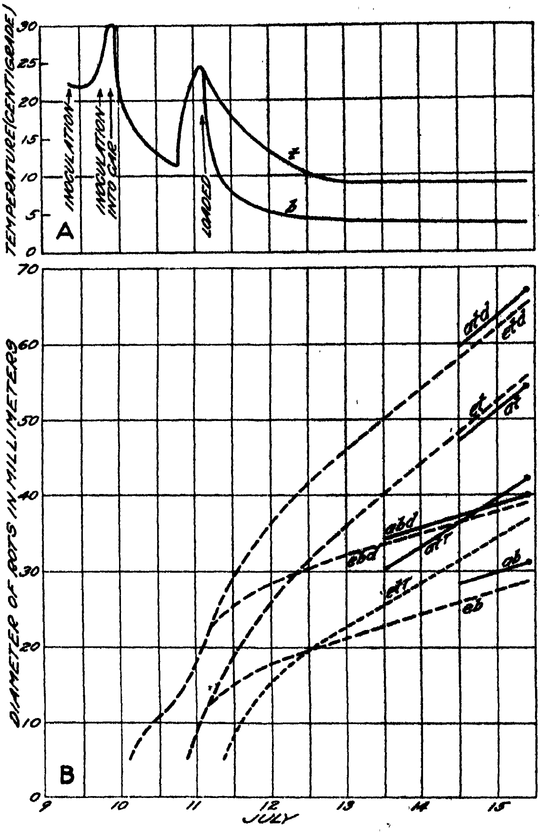

FIG. 24.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer in the same stack. B, development of rots (needle inoculations) on Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under conditions of curve A, *t*, with Monilia inoculations 6 hours before cooling; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 16 hours before cooling; *eb* and *ab*, under the conditions of curve A, *b*, with Monilia inoculations 6 hours before cooling; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 16 hours before cooling; *etr* and *atr*, under the conditions of curve A, *t*, with Rhizopus inoculations 6 hours before cooling

With Rhizopus needle inoculations on Elberta peaches the actual growth was behind the estimated in 3 instances, ahead of it in 1, and approximately the same in 11. With similar inoculations on Hiley peaches the actual growth was behind the estimated in 1 instance, ahead in 5, and approximately the same in 7. On a percentage basis, the actual results were behind the estimated in 20 per cent of the tests with Elberta and in 8 per cent with Hiley; and they were ahead of the estimated in 7 per cent of the tests with Elberta and 38 per cent with Hiley.

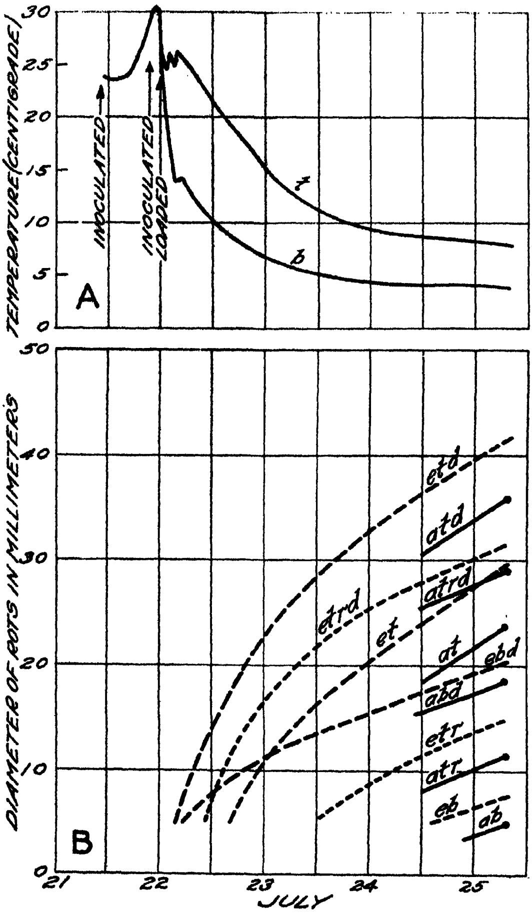

FIG. 25.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer of crates in the same stack. B, development of rots (needle inoculations) on Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under conditions of curve A, *t*, with Monilia inoculations 2 hours before loading; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 14 hours before loading; *eb* and *ab*, under the conditions of curve A, *b*, with Monilia inoculations 2 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 14 hours before loading; *etr* and *atr*, under the conditions of curve A, *t*, with Rhizopus inoculations 2 hours before loading; *etrd* and *atrd*, under the conditions of curve A, *t*, with Rhizopus inoculations 14 hours before loading

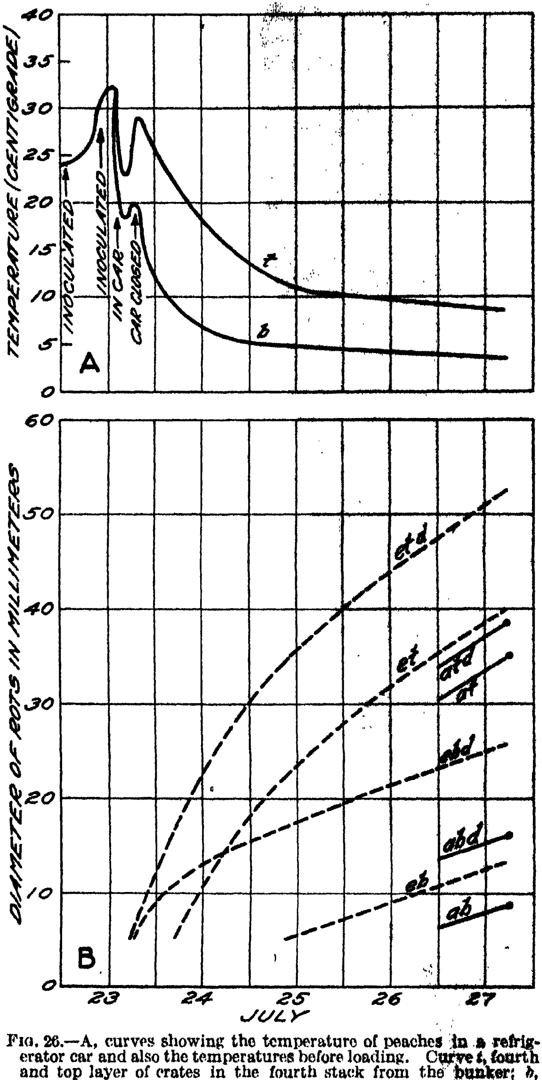

FIG. 26.—A, curves showing the temperature of peaches in a refrigerator car and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer of crates in the same stack. B, development of Monilia (needle inoculations) on rather green Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with inoculations 4 hours before loading; *etd* and *atd*, under the conditon of curve A, *t*, with inoculations 16 hours before loading; *eb* and *ab*, under the conditions of curve A, *b*, with inoculations 4 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with inoculations 16 hours before loading.

The results indicate that Elberta peaches are considerably more resistant than Hiley to both Monilia and Rhizopus rots. This is in agreement with market reports and with general opinion. It should be noted, however, that the data for the shipment described in Figure 26 account for much of the resistance to rot shown by the Elberta variety and that the peaches of this shipment were rather green. It was noticed in a number of experiments that greenness seemed to introduce a greater factor of rot resistance with Elberta than with other varieties. In the present studies Elberta peaches in a market-ripe condition and with a broken skin have shown but little more resistance to rot than Hiley, Belle, or Carman. It is the opinion of the writers that the greater freedom from rot found in shipments of well-matured Elberta peaches is largely due to a greater resistance to punctures and bruises.

TYPES OF INOCULATION

Several different types of inoculation with Monilia were tested. The results from the needle inoculations are shown in Figures 20 to 30, inclusive, and the results from other methods in Table 3.

TABLE 3.—*Effect of different types of Monilia inoculation on peaches shipped under different temperature conditions*

Treatment		Condition of fruit upon arrival					
Nature	Time before loading (hours)	Percentage of peaches affected		Average diameters of rots (millimeters)		Average number of rots per peach	
		Top of car	Bottom of car	Top of car	Bottom of car	Top of car	Bottom of car
HILEY PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 23							
No inoculation	-------	4.8	2.2	22.6	25	0.05	0.02
Rubbed (not smeared) with rotten peaches carrying but few spores	17	100	95.3	Total.	68	-------	-------
Do	2	100	26.7	45.6	12.4	-------	-------
Dusted with spores (no punctures)	17	87.5	13.9	51.2	32.3	-------	-------
Do	2	37.8	10.7	17.0	18.2	-------	-------
Dusted with spores after receiving 20 pin-point punctures to the peach	17	97.3	64.7	13.8	4.6	4.3	1.4
Do	2	84.2	9.1	3.1	2.3	2.3	.1
Wet peaches punctured and dusted as above	17	100	92.5	Total.	4.8	(a)	7.1
Do	2	100	38.5	17.3	1.7	3.8	.9
HILEY PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 24							
No inoculation	-------	7.1	2.8	22.8	14.9	0.08	0.03
Rubbed (not smeared) with rotten peaches carrying but few spores	6-32	80	52.9	37.8	29.7	-------	-------
As above, but with peaches that had received 20 pin-point punctures to the peach	6-32	100	100	Total.	31.8	-------	-------
Dusted with spores (no punctures)	16-42	30	29	23.8	19.8	-------	-------
Do	6-32	32.4	2	24.8	58.0	-------	-------
Dusted with spores after receiving 20 pin-point punctures to the peach	16-42	100	73.8	57.4	30.4	(a)	1.6
Do	6-32	97.4	76.9	10.8	7.6	3.5	2.4
Wet peaches punctured and dusted as above	16-42	100	100	Total.	58.8	(a)	(a)
Do	6-32	90	100	Total.	6.8	(a)	6.5
ELBERTA PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 25							
No inoculation	-------	1.1	0.9	28.8	22.5	0.01	0.01
Rubbed (not smeared) with rotten peaches carrying no evident spores	2	96.7	13.3	18.6	8.9	2.0	.1
Dusted with spores (no punctures)	20	59.3	0	12.3	0	.9	0
Do	2	37.5	0	7.4	0	.8	0
Dusted with spores after receiving 10 pin-point punctures to the peach	20	95.7	33.2	10.5	8.1	3.1	.6
Do	14	61.3	9.5	9.0	6.8	.9	.1
Do	2	38.6	3.0	6.3	6.2	.5	.03

TABLE 3.—*Effect of different types of Monilia inoculation on peaches shipped under different temperature conditions*—Continued

ELBERTA PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 26

Treatment		Condition of fruit upon arrival					
Nature	Time before loading (hours)	Percentage of peaches affected		Average diameters of rots (millimeters)		Average of number of rots per peach	
		Top of car	Bottom of car	Top of car	Bottom of car	Top of car	Bottom of car
No inoculation		2.6	0	21.5	0	0.03	0
Rubbed (not smeared) with rotten peaches carrying few if any spores	4	31.8	7.4	[a]		.6	.1
Dusted with spores (no punctures)	21	23.3	4.8	24.3	12.0	.3	.05
Do	15	6.9	3.5	8.6	11.8	.1	.05
Dusted with spores after receiving 10 pin-point punctures to the peach	26	71.9	46.4	17.5	10.2	1.4	1.0
Do	21	44.5	30.0	12.6	4.5	.7	.5
Do	15	85.2	10.5	18.4	10.6	2.6	.1
Do	4	16.7	0	13.2	0	.2	0

HILEY PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 30

Nature	Time before loading (hours)	Percentage of peaches affected: Top of car	Percentage of peaches affected: Bottom of car	Average diameters of rots (millimeters): Top of car	Average diameters of rots (millimeters): Bottom of car	Average of number of rots per peach: Top of car	Average of number of rots per peach: Bottom of car
CAR 1							
No inoculation		6	0	26.6	0	0.085	0
Dusted with spores after receiving 10 punctures each with No. 18 wire	2	100	7	15.3	16.0	5.9	.14
As above, but receiving 10 pin-point punctures each	2	95	12	17.8	8.7	4.2	.24
CAR 2							
No inoculation		8	1	32.0	22.5	.15	.04
Dusted with spores after receiving 10 punctures each with No. 18 wire	2	100	100	Total.	11.0	Total.	6.4
As above, but receiving 10 pin-point punctures each	2	100	78	Total.	12.7	Total.	2.3

[a] Rots confluent.

Needle inoculations gave practically 100 per cent infection in all cases. Of all the methods used they gave the largest rots at the end of the shipment thus indicating the shortest period of incubation.

Rubbing peaches with rotten fruit that carried few if any spores produced a high percentage of infection and a rapid rot, showing the serious results that may follow from allowing partly rotten peaches to come in contact with those that are intended for market.

Wetting the peaches before puncturing and dusting greatly increased both the percentage of infection and the size of the rots. This may have been partly due to a greater number of spores clinging to the wet fruit than to the dry, but the wetness probably also hastened germination and facilitated infection.

The rots on the peaches that were punctured and dusted without wetting averaged about one-third the size of the needle-inoculation rots, and as estimated by the growth values of Figure 2 were 2.5 to 3 days behind them in development.

The lead of the rots on peaches that were punctured before dusting over those that were dusted without intentional puncturing was in the number of the rots and not in their size. The same thing holds true in a comparison of the two types of dusted peaches with the untreated checks. In fact the rots resulting from dry-dust inoculations a few hours before loading averaged much smaller than those on the untreated fruit. This difference was evidently due to the fact that most of the rots on the untreated peaches had made an earlier start than those on the dusted peaches.

The peaches that were punctured and dusted had an average of about four and five-tenths times as many rots as those which were dusted without puncturing and more than one hundred and sixty times as many as those which were untreated.

The results as a whole show that spores carried on the surface of the fruit may be a cause of serious loss in transit, especially if the peaches have been punctured or otherwise roughly handled.

Spores were rarely produced by the rots during the transit period, even when the needle-inoculation fruit was delayed in loading, and it is safe to state that they were never produced in time to develop rots before the arrival of the shipment at its destination. All of the rots developed, therefore, were produced by the spores or mycelium carried by the fruit at the time of loading.

SPRAYED AND UNSPRAYED FRUIT

The peaches used in all the previous experiments had been sprayed according to prevailing orchard schedules, but the writers were fortunate in being able to obtain comparable lots of sprayed and unsprayed fruit from the experimental plots of John W. Roberts and John C. Dunegan at Fort Valley, Ga. The peaches described as unsprayed had received no fungicidal treatment whereas the sprayed fruit had been given the full spray-schedule treatment. The peaches were picked and packed under commercial conditions, apparently sound peaches only being included in the pack. One to three crates of peaches were used under each shipping condition. The results are shown in Table 4.

TABLE 4.—*Percentage of sprayed and unsprayed peaches affected with Monilia rot in transit*

[All fruit was apparently sound at the time of packing]

Variety	Year	Temperature as shown in—	Percentage of peaches affected with Monilia rot at the time of unloading			
			Top		Bottom	
			Sprayed	Unsprayed	Sprayed	Unsprayed
Yellow Hiley	1922		13.3	32.8	5.9	13.1
Hiley	1921		10.0	13.5	1.6	2.8
Elberta	1921	Fig. 21, A	27.3	91.1	5.3	39.9
Yellow Hiley	1922	Fig. 22, A	4.6	26.4	2.2	12.5
Do	1923	Fig. 27, A	11.8	31.7	6.3	12.5
Do	1923	Fig. 28, A	1.7	21.4	.5	7.9
Elberta	1923	Fig. 29, A	1.3	3.7	0	0
Hiley	1924	Fig. 23, A	4.8	16.7	2.2	11.7
Do	1924	Fig. 24, A	7.1	37.3	2.8	25.2
Elberta	1924	Fig. 25, A	1.1	12.0	.9	9.1
Do	1924	Fig. 26, A	2.6	42.2	0	13.1
Average			7.8	29.9	2.5	13.4

The unsprayed peaches developed far more brown rot in transit than the sprayed ones, in many cases eight to ten times as much. The average for 11 cars showed 7.8 per cent of the sprayed fruit in the top of the cars affected with Monilia rot, as compared with 29.9 per cent of the unsprayed fruit; and 2.5 per cent of the sprayed fruit in the bottom of the car was similarly affected, as compared with 13.4 per cent of the unsprayed fruit. The results show that the value of orchard spraying does not end in the orchard, but follows the fruit to its final destination.

In the top of the car the unsprayed peaches had practically the same percentage of rot as sprayed peaches that had been dusted (not punctured) with Monilia spores a few hours before loading, but in the bottom of the car the unsprayed peaches had more than three times as many rots as the comparable lots of dusted peaches. (Tables 3 and 4.) This contrast between the top and the bottom of the cars might be explained on the hypothesis that at the time of loading the unsprayed peaches carried fewer spores but more incipient rots than sprayed peaches which were dusted with Monilia spores; the temperature of the bottom of the car causing a greater relative decrease in infection than in the development of rots that had already made a start.

The results of the various experiments emphasize the importance of having the peaches as free as possible from spores as well as infections at the time they are loaded for shipment.

DELAYS BEFORE LOADING

A study of the results from Monilia needle inoculations (figs. 20 to 27, inclusive) shows that with this type of infection every hour of delay before loading puts the rots in the top of the car more than three hours ahead and those in the bottom of the car more than five hours ahead.

A similar study of the Rhizopus needle inoculations (Table 2, interpreted by Figures 2 and 4) shows that every hour of delay before loading put the rots in the top of the car nearly five hours ahead. The delays also greatly increased the percentage of infection. A delay of 10 to 15 hours sometimes meant three to five times as many infections, and in other cases it meant the difference between no infection and 20 to 100 per cent of infection

The effect of delay upon the Monilia dusting inoculations was also significant, but not easy to summarize. A delay of a few hours before loading often meant two to three times as many rots at the end of the shipment, sometimes 10 to 12 times as many. Where there was little contrast in the number of rots, the effect of the delay was usually evident in their size, the rots on the delayed fruit often being several times as large as those on the fruit that was more promptly loaded.

COOLING OF THE CAR

A comparison of Figures 20 to 30, inclusive, shows considerable variation in the cooling of the different cars. Since the inoculations and the fruit in the different cars were not usually comparable, it is impossible to accurately interpret these differences in cooling in terms of actual rot development at the destination. A comparison can be made, however, on the basis of the values of Figures 2 and 4.

A study of the results reported in Figures 25 and 30 shows that the fruit of the two shipments had practically the same temperature at the time of loading, but that at the end of 24 hours, the top of the car described in Figure 25 was down to 15° C. while the top of one of the cars described in Figure 30 had reached only 22.5°. According to the values of Figures 2 and 4 this difference in temperature meant that the rots in the top of one car were developing nearly twice as rapidly as those in the other. In the shipments described in Figures 24, 27, and 28, the temperature in the top of the car at the end of 24 hours was still lower than that of Figure 25, indicating a still greater check in the development of rots.

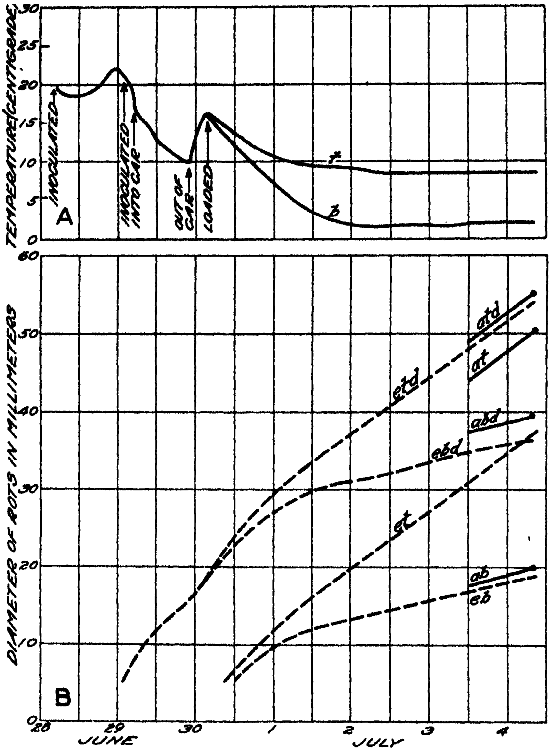

Fig. 27.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. The experimental peaches were held inside the refrigerator car the night previous to final loading, and the temperature curves probably do not show the condition of the general load. Curve *t*, fourth and top layer in the fourth stack of crates from the bunker; *b*, bottom layer of the same stack. B, development of Monilia (needle inoculations) on Yellow Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with inoculations 3 hours before cooling; *etd* and *atd*, under the conditions of curve A, *t*, with inoculations 24 hours before cooling; *eb* and *ab*, under the conditions of curve A, *b*, with inoculations 3 hours before cooling; *ebd* and *abd*, under the conditions of curve A, *b*, with inoculations 24 hours before cooling

The most interesting contrasts in cooling, however, are to be found in a comparison of the top and the bottom of the same car.

CONTRAST BETWEEN THE TOP AND THE BOTTOM OF THE CAR[5]

If the temperature curves of Figures 20 to 26 and 28 to 30, inclusive, are interpreted in terms of the work values or time-temperature units

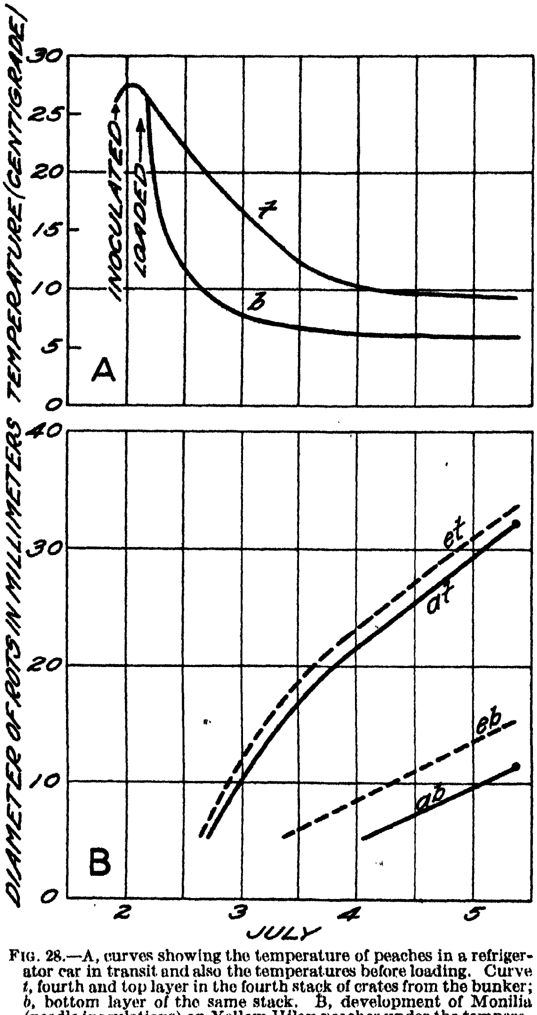

FIG. 28.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer in the fourth stack of crates from the bunker; *b*, bottom layer of the same stack. B, development of Monilia (needle inoculations) on Yellow Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4. Curves *et* and *at* give the results under the conditions of curve *t*; *eb* and *ab* under the conditions of curve *b*. All inoculations were made five hours before loading

of Figure 4 with the development of a 5-mm. rot represented by 100, it is found that for Monilia needle inoculations the temperatures recorded for the bottom of the cars would have an average rating of 105, whereas those for the top of the cars would have an average rating of 266, or two and fifty-three hundredths times as high as that for the bottom of the cars. If instead of the needle-inoculation curve, the Monilia dusting and puncture curve is used, the ratings are about 53 for the bottom of the cars and 133 for the top of the cars. These ratings mean that in the bottom of the car Monilia spores pushed into the flesh of the peach in quantity would barely have time to produce a visible rot during the actual transit period, and with cooling such as is shown in Figures 22, 24, and 29 they would not have time to do so; and they also mean that spores lodged on the skin of a peach, even at a puncture, could rarely, if ever, produce a rot during such transit periods as are described in Figures 20 to 30, inclusive. In contrast to this, the ratings for the top of the car would give two and

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[5] Reference is here made to cars loaded four layers high with six-basket carriers.

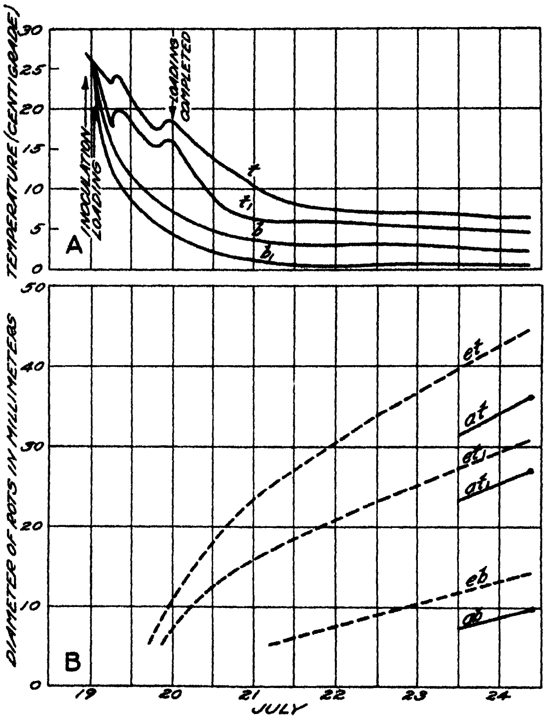

FIG. 29.—A, curves showing the temperature of peaches in a refrigerator car in transit. Curve *t*, fourth and top layer at the center of the car; *b*, bottom layer, center of the car; t_1, fourth and top layer next to the bunker; b_1, bottom layer next to the bunker. B, development of Monilia (needle inoculations) on Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4. Curves *et* and *at* give the results under the conditions of curve A, *t*; et_1 and at_1 under the conditions of curve A, t_1; and *eb* and *ab* under the conditions of curve A, *b*. There was no growth under the conditions of curve A, b_1. All inoculations were made two hours before loading

six-tenths times as many work units as are required for Monilia needle inoculations to produce visible rots and one and one-third times as many as the average required for the production of rots by dusting spores on punctured peaches.

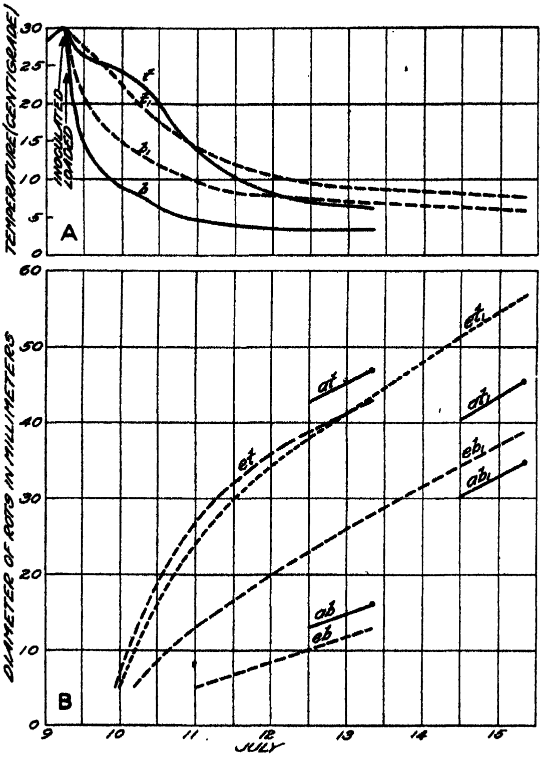

FIG. 30.—A, curves showing the temperatures of peaches in two refrigerator cars in transit and also the temperatures at loading. Curve *t*, fourth and top layer of crates in the fifth stack from the bunker; *b*, bottom layer of crates in the same stack of the same car; t_1, third and top layer of baskets two stacks from the door of the second car; b_1, bottom layer of baskets of the second car in front of the door; t_1, like previous test lots, was in the center of the car crosswise, but b_1 was in the outside row. B, development of Monilia (needle inoculations) on Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination and the lines connecting with these dots trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4. All inoculations were made one hour before loading: Curves *et* and *at* give the results under the conditions of curve A, *t*; *eb* and *ab*, under the conditions of curve A, *b*; et_1 and at_1, under the conditions of curve A, t_1; eb_1 and ab_1, under the conditions of curve A, b_1

With Rhizopus the contrasts between the top and the bottom of the car would be even greater than with Monilia, but not usually so evident in practical results, since in many cases the temperature of the top as well as the bottom of the car was too low for the production of rots during the transit period, even from needle inoculations.

The temperature contrasts between the top and the bottom of the car are composed of two factors, one the temperature level during the last days of the shipment and the other the length of time required to reach this level. The average temperature for the top of the car at the destination was about 4.5° C. higher than that for the bottom, but there was a period of 24 to 36 hours after the cars were closed when the top was 7° to 14° warmer than the bottom. According to the temperature values of Figure 4, nearly 60 per cent of the damage done by Monilia during the given transit periods in either the top or the bottom of the car was accomplished during the first 36 hours after the car was closed. Also, 60 per cent or more of the gain that the rots in the top of the car made over those in the bottom during the transit periods was accomplished during the first 36 hours, and with the shorter and more typical shipments the gain in the top of the car over the bottom was nearly twice as great during the first 36 hours as during the remainder of the period of shipment. The transit period could have been continued one to five days longer, or an average of about 41 hours longer, at the temperatures at which the shipments finished, before the damage resulting from the latter part of the shipment (about 103 hours) would have equaled that produced during the first 36 hours.

In the actual shipping tests, the inoculations were usually made several hours before the fruit was loaded, thus making the final results the combined effect of the car-temperature conditions and the delay in loading, and as previously pointed out (p. 536), the additive effect of the delay was sometimes more significant in the bottom of the car than in the top. In spite of this fact the actual results seem to fully support the differences outlined above.

A study of the growth curves in Figures 20 to 30, inclusive, shows that with the Monilia needle inoculations the actual work units accumulated in the top of the car averaged about 2.5 times as great as those for the bottom of the car, which is in agreement with the estimated values reported above. It should also be noted (based on growth curves continued backward) that, with inoculations made 1 to 6 hours before loading, the rots in the top of the car appeared 17 to 65 hours earlier than those in the bottom of the car and averaged about 42.5 hours earlier.

With Rhizopus needle inoculations made 1 to 6 hours before loading (Table 2), practically no rots started in the bottom of the car, whereas with the exception of the shipments of Figures 28 and 29 a good growth was made in the top of the car.

With Monilia dusting inoculations the contrast in the size of the rots in the top of the car and in the bottom was not so great as with the needle inoculations, but the contrast in the number of rots was especially significant. With the peaches that were punctured and dusted 1 to 6 hours before loading, there was an average of about fifty times as many rots in the top of the car as in the bottom at the destination of the shipment, and with peaches punctured and dusted 14 to 21 hours before loading there was an average of about 4 times as many rots in the top as in the bottom.

Peaches dusted with Monilia spores without puncturing developed about twenty times as many rots in the top of the car as in the bottom.

The regular commercial pack of fruit (Tables 3 and 4) averaged about four times as many rots in the top of the car as in the bottom.

The greater difference between the top and the bottom of the car in the case of peaches punctured and dusted 1 to 6 hours before loading than with those similarly treated 14 to 21 hours before loading would naturally be expected, owing to the fact that the delay gave the rots a chance to start before loading, and they were then able to continue to develop at the bottom as well as at the top of the car. The greater contrast with the dusted peaches than with the commercial pack probably has a similar explanation and is to be attributed to the fact that with the dusted peaches any infections already established are greatly outnumbered by the potential infections, whose development is determined by the temperatures of the transit period.

In commercial shipments the writers have frequently observed that while there are many more rots in the top of the car than in the bottom, there may be no corresponding contrast in the size of the rots. This is apparently due to the fact that the rots in the bottom of the car are confined largely to those that have made some start before loading, whereas many of those in the top of the car have developed during the transit period.

The various differences reported above are between the top and bottom carriers midway between the bunker and the door and do not show the extreme contrasts of the car as found between the bottom layers at the bunker and the top layers at the door. They are sufficient, however, to show the extreme variations in rot production that are often found in the same car.

SUMMARY

A study has been made of the relation of temperature, spraying, and type of infection to the transportation rots of peaches, and an attempt is made to equate the different temperature and infection values.

On the basis of temperature values developed and with the temperature conditions known, it has been found possible to picture the approximate course of development of the rots.

Monilia and Rhizopus rots increased in diameter at an approximately uniform rate in the different stages of development, the diameter of the rots thus becoming a fairly accurate basis for temperature comparisons. For the purposes of the present studies neither the area, nor the volume of the rot, nor the weight of the rotten tissue could be used as a basis for temperature equivalents.

With both Monilia and Rhizopus, low temperatures had a relatively greater inhibiting action upon development during the incubation period than during the later growth. With both incubation and growth a 5° C. change in temperature had a greater effect at the lower temperatures than at the higher ones (experiments ranging from 0° to 30°).

With inoculations made by dusting spores over punctured peaches, the incubation period for Monilia was about twice as long as with needle inoculations, and with spores dusted over apparently sound peaches the incubation period was still further prolonged. A similar contrast was found with Rhizopus, except that the fungus was apparently unable to penetrate the sound skin of market-ripe peaches.

The rots from Monilia dust inoculations continued to appear over a long period of time, whereas those from needle inoculations started off practically together. With Rhizopus there was considerable spread of the infection period in the case of both needle and dust inoculations.

When once established, the Monilia and Rhizopus rots that had resulted from dust inoculations enlarged as rapidly as those from needle inoculations.

Peaches that were punctured and then dusted with Monilia spores developed about four and five-tenths times as many rots as those that were dusted without puncturing and one hundred and sixty times as many as untreated peaches.

Apparently sound unsprayed peaches developed about four times as many rots in transit as similar sprayed peaches.

With dust inoculations and with fruit that had not been inoculated the contrast between the top and the bottom of the car and between delayed and immediate cooling was in the number of rots rather than in their average size. This was apparently due to a delay and spread in the infection period resulting from the lower temperatures and the more rapid cooling.

In the production of Monilia rots the temperatures of the top of the car had about two and five-tenth times the value of those in the bottom of the car, and about 60 per cent of this contrast and 60 per cent of the growth value in both the top and the bottom of the car were developed during the first 36 hours after the car was closed.

Monilia needle-inoculation rots appeared 17 to 65 hours earlier in the top of the car than in the bottom and averaged about 42.5 hours earlier. Peaches that were punctured and then dusted with Monilia spores 1 to 6 hours before loading, developed about fifty times as many rots in the top as in the bottom of the car, and peaches similarly treated 14 to 21 hours before loading developed about four times as many rots in the top as in the bottom of the car.

DEVELOPMENT OF THE BACTERIA CAUSING WILT IN THE ALFALFA PLANT AS INFLUENCED BY GROWTH AND WINTER INJURY[1]

By Fred Reuel Jones[2]

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INTRODUCTION

Bacterial wilt of alfalfa has been closely linked with winter injury of that plant in various ways from its earliest history to the present time. Before bacterial wilt was recognized as a disease caused by a specific organism (*Aplanobacter insidiosum* L. McC.), its symptoms were often ascribed to injury incurred by the plant during an unfavorable winter. After the bacterial origin of wilt was recognized, some of the most destructive epidemics of the disease were observed to follow the partial winterkilling of fields and the severe injury of the surviving plants in which wilt later appeared. This sequence suggested that winter injury made plants susceptible to wilt, though the manner in which this was brought about was not obvious from observation in the field. During the early part of the study of the bacterial disease reported by McCulloch and the writer in a previous paper,[3] no relationship between it and winter injury was discovered.

Later, however, the method found to stain bacteria in the host tissue proved equally satisfactory for the study of the pathological histology of winter injury. Moreover, lesions resulting from freezing often so strikingly resembled those caused by bacteria that it became necessary to study the pathological histology of winter injury before that of wilt could be distinguished with certainty in all cases. This study of winter injury of alfalfa has been published in the second paper[4] in this series. In the course of the comparative study of the two diseases evidence was obtained which indicated that the action of frost upon the tissues of diseased plants served to release the bacteria for distribution in the production of epidemics and also furnished wounds through which they enter and infect plants. Thus the connection between bacterial wilt and winter injury appears to be, locally at least, more important than accidental association or superficial resemblance. It remains, therefore, in this, the third paper in the series, to describe the development of the bacteria in the host plant and the pathological conditions resulting therefrom, and to present evidence that winter injury may provide favorable conditions for epidemics of the disease in infested fields. A brief article on bacterial wilt and winter injury has already been published.[5]

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[1] Received for publication July 19, 1928; issued December, 1928. Cooperative investigations between the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Experiment Station of the University of Wisconsin.

[2] The writer is indebted to many persons for aid in locating and collecting material used in the preparation of this paper, and for suggestions of many kinds. J. L. Weimer, stationed at Manhattan, Kans., has spent much time assisting the writer in collecting material and in interpreting field conditions in Kansas. The drawing presented as Figure 1 was prepared by Dr. Vladimir Skoric.

[3] Jones, F. R., and McCulloch, L. A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY APLANOBACTER INSIDIOSUM L. M'C. Jour. Agr. Research 33: 493–521, illus. 1926.

[4] Jones, F. R. WINTER INJURY OF ALFALFA. Jour. Agr. Research 37: 189–212, illus. 1928.

[5] Jones, F. R., and Weimer, J. L. BACTERIAL WILT AND WINTER INJURY OF ALFALFA. U. S. Dept. Agr. Circ. 39, 8 p., illus. 1928.

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Key No. G-642

METHODS OF STAINING AND COLLECTING MATERIAL

The method of staining that made possible clear distinction between pathological conditions caused by bacterial wilt and winter injury in alfalfa plants was based upon the fact that the bacteria are strongly Gram-positive. The bacteria in the tissue were stained by Gram's stain, usually prepared by Sterling's modified method [6] and the tissue elements were differentiated by staining with safranin and orange G without interfering with the brilliant-blue stain in the bacteria. The Gram's stain served also to stain in a characteristic manner a deposit of material that follows winter injury in comparatively young tissue; and thus, even when the bacteria occur in the same sections with injury, the two pathological conditions are easily distinguished. Details of the staining method have been published previously [7] and need not be repeated here.

After this method of staining had been developed and tested in January, 1926, it was at once apparent that it was more serviceable as a means of determining the presence of the pathogenic bacteria in plants in which they were few in number or mixed with other bacteria than the usual method of identifying them from agar plates prepared from the material. From this time onward, whenever doubtful cases of bacterial wilt were encountered in fields, portions of the suspected plant were fixed in formal-acetic alcohol for future cytological examination. In case it was not convenient to fix the material in the field, the dried roots were sometimes soaked or boiled in water later and fixed and sectioned in the usual manner with satisfactory results in so far as the staining of the bacteria was concerned. Thus it was possible to examine the relation of the bacteria to the host tissues in dried roots collected in previous years.

During the spring of 1926, when the most extensive collection of wilt-infected plants began, a great deal of winter injury was found in both Wisconsin and Kansas, where the larger number of collections was made. At this time the necessity for the comparative study of the pathological histology of winter injury and of wilt became apparent, and collections of portions of plants appearing to show one or both of the diseases were begun for the purpose of checking field observations with trustworthy cytological evidence. Collections were continued during 1927. Usually the upper portion of the root close below the crown was taken in the earlier collections, but later, when the necessity for learning more of the development of the bacteria in the crown was recognized, crowns and stems were collected also. At the time of writing this paper about 200 collections of plants showing wilt, winter injury, or both have been assembled, chiefly from the central Mississippi Valley, and more or less completely examined.

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[6] ZINSSER, H., and RUSSELL, F. F. A TEXTBOOK OF BACTERIOLOGY. Ed. 5, pp. 120-123. New York and London, D. Appleton and Company. 1922.

[7] JONES, F. R. Op. cit.

RELATION OF VISIBLE DISCOLORATION OF WOOD OF ROOT TO LOCATION OF BACTERIA

In the previous paper[8] the most characteristic symptom of the bacterial disease found at all times in the root was described as a yellow discoloration of the outer part of the wood that increased in extent as the disease progressed and that indicated, in so far as could be determined by the isolation of the bacteria on agar plates, the tissue actually invaded by the bacteria. The method of staining the bacteria in the tissue noted here has disclosed the relation of the visible discoloration to the actual location of the bacteria and has shown that the bacteria are not usually as extensively distributed in the wood as the discoloration has suggested. First of all, the discoloration is not due exclusively to the yellow gumlike material previously described in the invaded vessels. Wood may be discolored even before the gum has appeared. This is best seen in newly infected plants. In one collection of recently infected plants showing stripes of pale-yellow color beneath the bark of the upper part of the root, the bacteria were found only in two or three vessels at the centers of the narrower stripes. In such cases the color had diffused to a considerable distance from the bacteria. The solubility of this coloring material was indicated by the fact that the color disappeared after the exposed wood had been soaked in water. This soluble stain appears to be present in varying quantities in the wood of the root, and it may diffuse long distances in badly diseased plants toward the center of the root, unaccompanied by any migration of the bacteria in that direction. This stain is not conspicuously present in stems.

Just as the soluble stain was found to diffuse in the wood far beyond the bacteria, so the yellow gumlike material of undetermined chemical character was found in vessels beyond those entered by the bacteria. The extent of gum formation varies with the location and abundance of bacteria. Usually vessels of autumn wood invaded by bacteria are completely filled with them, and gum is formed slowly and may not extend far beyond the invaded vessels. In summer wood, bacteria may not be abundant in the invaded vessels, forming only thin layers along the sides and between the inner thickenings. Here gum formation may appear in vessels contiguous to those invaded even before it is discernible in invaded ones, and it may extend to all contiguous vessels in the group of which the invaded vessel forms a part, or even to vessels separated by a few small parenchymatous cells, but usually not to those separated by fibers. Thus, one invaded vessel may induce gum formation in five or six others, and a few bacteria well distributed in the vascular system may cause a relatively enormous obstruction of vessels.

The origin and manner of formation of the gum are not easily discovered in sections. It appears to be thin and flocculent in character at first and becomes more firm with age, until at length it is hard and resinous and shrinks away from the walls of well-filled vessels. Sometimes it does not fill the lumen of the vessel but leaves an open passage in the center. When it fills vessels invaded by bacteria it does not usually engulf the bacteria but crowds them into vacuole-like pockets. (Fig. 1.) From such observation of the behavior of the gumlike material as has been made thus far, it appears to be a product of the alfalfa plant and not of the bacteria.

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[8] JONES, F. R. and McCULLOCH, L. Op. cit.

The invasion of the phloem or of the young stems by the bacteria is not accompanied by the conspicuous and characteristic color changes that accompany invasion of the wood. For this reason the extent and importance of phloem invasion in causing the death of plants was not recognized in the earlier study of this disease. The invaded phloem may be water-soaked in appearance and in time may take on a yellow color, caused apparently by the development of the soluble yellow stain, which usually diffuses so far from the bacteria that it is of little aid in determining precisely their location. The stain has not been seen in young stems. The extent of bacterial invasion outside the wood of the root can usually be determined only by the microscopic examination of stained sections of tissue.

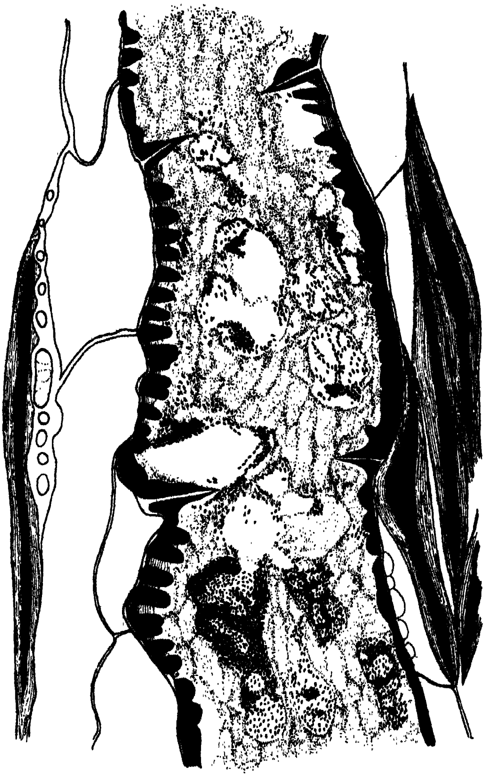

FIG. 1.—Longitudinal section of a vessel in the summer wood of an alfalfa plant infected with wilt. This material appears to have been fixed after gum formation was complete but before the gum had begun to shrink away from the vessel wall. The bacteria are found first characteristically along the vessel wall. The gum, formed presumably by the surrounding cells, has pushed nearly all of the bacteria away from the wall. × about 800

PROGRESS OF BACTERIA IN HOST PLANT FROM INFECTION TO DEATH OF PLANT

INVASION OF WOUNDS AND ENTRY OF VASCULAR SYSTEM

The infection of the plant by the bacteria producing wilt is, so far as is known, wholly through wounds. The wounds may be of such character that entry may be made in two very different ways. The bacteria may be introduced directly into the vascular system through the cut ends of stems, or they may enter between cells of parenchymatous tissue through which they force their way to the vessels. The first method of infection, while effective in artificial inoculations, appears to be unusual in the field. In nature it appears that the bacteria usually reach the vessels through parenchymatous tissue. Wounds in the root, through which the bacteria may gain entrance, expose phloem parenchyma, and, similarly, wounds in the crown stems, at least those resulting from winter injury, also expose phloem parenchyma after the sloughing off of the cortex. Inasmuch as the behavior of the bacteria in the tissues exposed by wounds, whether in roots or stems, is essentially the same, invasion through wounds in roots will be traced for present illustrative purposes.

Wounds in the phloem when made artificially may in old roots penetrate only tissue several years of age that is greatly crushed from internal pressure. Such wounds if extensive do not usually remain superficial. The internal pressure that crushed the outer tissue, no longer opposed by the encircling bark, often leads to a cracking of the phloem along a phloem ray nearly down to the cambium, or at least permits the enlargement of ray cells and the opening of large intercellular spaces as the tissue is pulled apart by tangential expansion. Cork cambium formed to protect the wound may be broken again and again because of insufficient elasticity in this direction. Thus wounds even when superficial often expose sooner or later the deeper active layers of phloem.

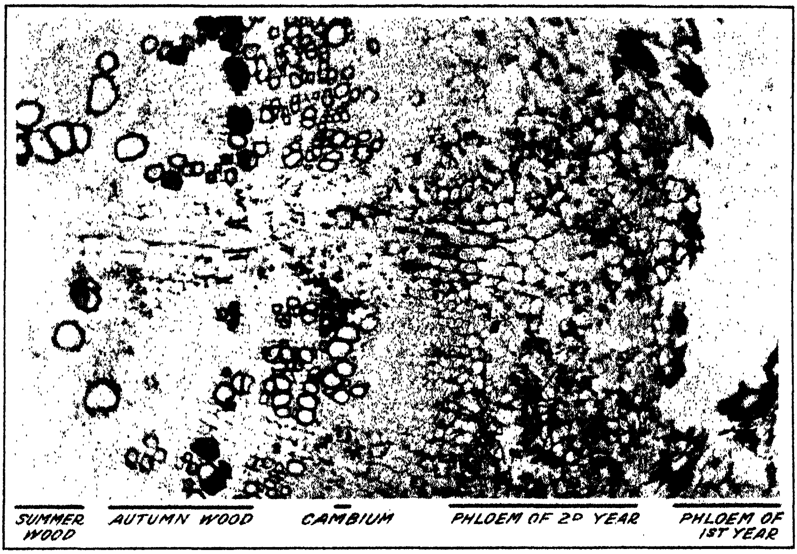

FIG. 2.—Photomicrograph of a portion of a stained cross section of a 2-year-old alfalfa root through a wound from which the bacteria producing wilt entered the vessels of the underlying vascular bundles. The bacteria are represented by the black masses in the vessels and between the parenchymatous cells. The black lines passing from the wound in the phloem along the ray cells through the interfascicular cambium into the xylem represent the course taken by the bacteria in entering the vascular bundles. In the central xylem ray the bacteria have passed a short distance into the zone of summer wood. At the right the bacteria have caused collapse of immature cells near the youngest vessels invaded. Some of the finer lines of bacteria between cells and scattered bacteria clinging to the inner walls of vessels that are not largely plugged are not shown in the photograph. The root used in this artificial inoculation was transplanted from the field inoculated November 3, 1925, and fixed January 26, 1926. × 76

When wounds are made in comparatively young phloem, as shown in Fig. 2, and the parasitic bacteria are brought in contact with such wounds, infection takes place readily. The precise manner in which the bacteria first make their way into the intercellular spaces of the parenchymatous tissue is a matter of conjecture. It is not improbable that they are carried into the intercellular spaces by capillary water, as in the following experiment: When the outer phloem of roots of growing plants is sliced off with a sharp knife under a bacterial suspension and the cut tissue is immediately fixed and stained, the bacteria are found in the larger intercellular spaces of the phloem to a depth of two or three cells from the surface. Apparently this tissue absorbs water strongly, and the bacteria are carried into the larger intercellular spaces with the water. The entrance of the bacteria into the plant tissue through natural wounds may take place in the same manner.

From the place of entry the bacteria spread through the larger intercellular spaces in all directions. Progress is most rapid along the phloem rays to the interfascicular cambium, which, unlike the fascicular cambium, is almost always composed of comparatively large cells with intercellular spaces. It may be noted here, however, that the bacteria are not limited to intercellular spaces. In certain young but fully expanded tissue the bacteria are often found surrounding cells in a thin layer scarcely more than one bacterial cell in thickness, as though they had grown through the middle lamella when it was in a plastic condition. Solution of the lamella is not evident. The interfascicular cambium does not usually appear to be damaged by the passage of the bacteria. In some cases it seems to have resumed growth after the bacteria have passed through it and to have separated the invaded parenchyma of the phloem from that in the wood with uninvaded tissue.

After passing the interfascicular cambium, the bacteria continue along the wood ray through about half the last annual increment laid down at the time the bacteria pass the cambium, unless, as sometimes happens in the spring, the deeper ray tissue has been previously split apart by winter freezing. Even when passage along rays is thus facilitated by the physical separation of cells, the bacteria do not usually develop abundantly in the older wood.

As soon as the bacteria have passed the cambium they begin to spread tangentially along the middle lamella between the parenchymatous cells of the vascular bundles until they reach the vessels. Occasionally they advance through immature cells close inside the fascicular cambium, causing the disintegration of these cells and the formation of bacterial pockets contiguous to young vessels. (Fig. 2.) The outer wall of the vessel appears to offer no resistance to the entry of the bacteria.

The bacteria do not continue to spread indefinitely among the cells of either phloem or xylem from the point of infection. Their spread seems to be slowed up largely by the growth from the cambium and the maturing of the tissue in which they are located during their slow advance. The relation of growth of the bacteria to the maturity of the tissue will be discussed in a later section.

DISTRIBUTION OF THE BACTERIA THROUGH THE VASCULAR SYSTEM

Soon after the bacteria have entered the vessels of two or three of the bundles in the manner shown in Figure 2 they are found in corresponding vessels in all the bundles around the circumference of the root and far up the stem as well as down the taproot. This comparatively rapid spread of the bacteria in the plant is one of the most important phases in the development of the disease, and therefore the manner in which it is brought about deserves special attention. Unfortunately, it is very difficult to follow it by direct observation.

There are three possible routes by which the bacteria may pass from vessel to vessel: First, by open passages between vessels; second, by penetration of the thin middle lamellalike walls separating contiguous vessels; and, third, by passing through the middle lamellae of walls of parenchymatous cells, separating vessels precisely as in the first invasion of vessels described.

DISTRIBUTION THROUGH OPEN CHANNELS

The open communications between vessels through which bacteria may pass without actually penetrating cell walls will first be examined. In a vertical direction in root and stem long open communicating vessels are easily demonstrated. If taproots of vigorous plants several years of age are cut with a razor under india ink in suitable dilution, the ink will often pass up or down from the cut 6 to 7 cm. in half an hour. In this way open communication through vessels is easily demonstrated from stems through crowns to the root. The extreme length to which these open passages may reach has not been determined, but at least they are sufficiently long to account for much of the vertical distribution of the bacteria in the plant through open channels alone.

This method of demonstrating the length of open vessels in the alfalfa plant reveals an added fact that is of interest here as well as in a later connection. The vessels through which the living plant will draw the ink to considerable distances when a cut is made under the ink suspension are located exclusively in the outer part of the root in the precise region where the bacteria distribute themselves whether they enter the vessels through wounds or are introduced like the ink through cut vessels. This experiment has been repeated during the summer, and, though the results show some variation, the conducting vessels demonstrated in this way are usually within one-half of a year's growth from the cambium.[9] The small vessels of autumn wood tend to retain conductivity slightly longer than the larger ones of the midsummer wood, and thus late in the summer when the early summer wood fails to draw the ink the wood of the previous autumn may still draw it a long distance. Since no obstructions have been found in the vessels that do not draw the ink, the assumptions made by MacDougal et al.[10] as a result of similar experiments with trees seem valid here; namely, that the vessels that fail to conduct the ink for long distances are in fact filled with air, not with water. Thus the fact that the inner vessels of infected plants never become filled with bacteria may be due not only to the inability of the bacteria to traverse the parenchyma far enough to reach them, but also to the absence of water by which the bacteria may be distributed through them.

Lateral openings in a tangential direction between vessels in sufficient number and of suitable arrangement to insure the open-channel distribution of the bacteria around the entire circumference of the

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[9] When these experiments were repeated in 1928 at Madison, Wis., many vessels in the spring wood of that year were found open and filled with water in the autumn in nearly all plants. Thus, contrary to findings in the previous year, the entire ring of 1928 wood retained its ability to conduct water throughout the growing season. Unfortunately, no inoculations were made at suitable dates to determine whether infection in the autumn could be followed by distribution of the bacteria through all the vessels actually filled with water. It seems unlikely that this would have happened, and therefore the conclusions drawn from the earlier observations are retained until they can be corrected, if necessary, by future work.

[10] MACDOUGAL, D. T., OVERTON, J. B., and SMITH, G. M. THE GAS-WATER SYSTEMS OF CERTAIN WOODY ESTMS. (Abstract) Amer. Jour. Bot. 14: 622-623. 1927.

root are not so easily demonstrated. However, open communication between vessels can be found. The vascular bundles, especially in the upper part of the root, pursue zigzag courses, joining lattice fashion around the wood rays. At the point of junction these rays when seen in cross section are marked by greater width, and in these wide places the converging vessels are often arranged side by side in rows across the bundle. These rows of vessels may fail to complete the contiguous lateral walls, thus leaving an open channel between them formed in precisely the same manner as the openings between the vessel segments. Sometimes three or four vessels converging from the two bundles have a common communicating channel in the same horizontal plane. (Fig. 3.) Whether or not all vessels communicate in this manner has not been determined. Each bundle converges with another bundle in the vascular net at each 2 or 3 mm. of its length. If each vessel has, as seems likely from the previous experiments, an open channel at least 6 cm. long, it passes in the course of its length through at least 20 vascular junctions where connections with other vessels may take place. In diseased plants masses of bacteria have been found connected from one vessel to another through such openings, and it appears that the bacteria are distributed around the circumference of the roots to some degree if not entirely through these open vascular connections.

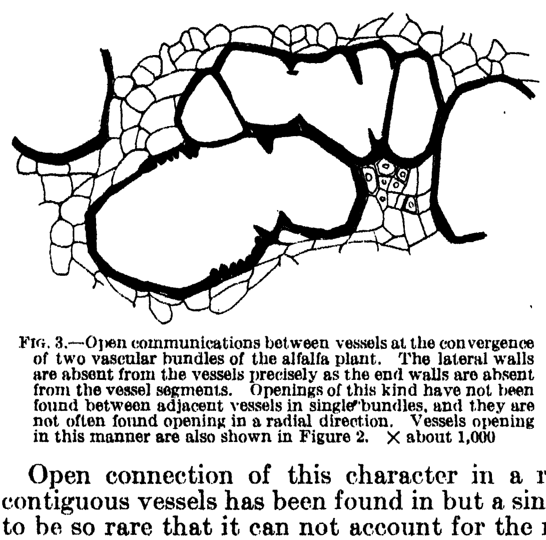

FIG. 3.—Open communications between vessels at the convergence of two vascular bundles of the alfalfa plant. The lateral walls are absent from the vessels precisely as the end walls are absent from the vessel segments. Openings of this kind have not been found between adjacent vessels in single bundles, and they are not often found opening in a radial direction. Vessels opening in this manner are also shown in Figure 2. × about 1,000

Open connection of this character in a radial direction between contiguous vessels has been found in but a single instance. It appears to be so rare that it can not account for the radial spread of the bacteria into new wood produced by the cambium.

Distribution by Passage Through Middle Lamellae of Walls of Contiguous Vessels

Inasmuch as it has been shown that the bacteria pass readily between parenchymatous cells and also appear at times to pass between vessels, it may seem highly probable that they can pass from one vessel to another contiguous vessel by penetrating the lamellae separating their lumina wherever the open spaces between the inner thickenings are opposed. A clear demonstration of this seemingly possible method of distribution has not yet been made. Certainly the bacteria do not always pass readily from one vessel into contiguous vessels, though it is probable that they do so in young tissue. Again and again in longitudinal sections bacteria are found confined to a single vessel, though it may be in contact with several others. Inasmuch as this method of passage of the bacteria from vessel to vessel has not been demonstrated and does not seem necessary to explain the distribution of the bacteria, it will not be discussed further.

PROGRESSIVE INVASION OF YOUNG VASCULAR TISSUE BY ADVANCING THROUGH PARENCHYMATOUS TISSUE

In the microscopic sections made from the upper part of the taproot of old diseased plants the parasitic bacteria may be found distributed through several annual rings of wood; but only rarely does a section reveal how the bacteria may have passed from the early inner wood to the more recent outer wood. The bacteria appear to invade new wood by advancing through young parenchymatous tissue chiefly young wood ray cells inside the cambium and entering the bundle and the vessels as soon as they have completed growth. The adjustment between host and parasite in this process may be so well made that the bacteria advance through the plant for several years without killing it. The essential features of this invasion may be seen in Figure 2, which shows the invasion of the vascular system by bacteria entering through a wound. In this figure the first vessels entered by the bacteria were undoubtedly the inner vessels showing plugging from the bacterial mass and gum. This probably took place before the outer vessels shown in the photograph were fully differentiated and developed. As these vessels reached mature size, the bacteria, following between the expanding interfascicular cells, were at all times ready to enter the bundle as soon as its maturity permitted, and thus the vessels were invaded in succession. Nearly all of the vessels in this younger wood are actually invaded and along their inner walls have bacteria too few in number to show in the photograph. It will be noted that at the right of the central ray the bacteria have invaded immature fascicular tissue, causing its collapse and the formation of bacterial pockets adjoining the invaded vessels. Bacterial pockets of this kind are not always formed in this situation and the bacteria may continue to develop between the cells close behind the interfascicular cambium for several years, invading successive layers of wood without producing much visible disorganization of the tissue and without inciting the tissue to inclose the invaded area with dividing cells as in a wound. The invasion of new wood does not always continue indefinitely at the place where the bacteria entered, but it may proceed at other places in the crown where bacteria have entered parenchyma from vessels. This will be discussed further in connection with the description of the progress of the bacteria through the parenchymatous issue.

INVASION OF PARENCHYMATOUS TISSUE

Certain characteristics of the invasion of parenchymatous tissue by the parasitic bacteria have been discussed. It has been noted that the bacteria progress more rapidly along the larger intercellular spaces; that they flourish best in comparatively young tissue but do not necessarily enter undifferentiated tissue near the fascicular cambium; and that the bacterial advance is very slow in comparison with that in most bacterial diseases—so slow that growth and maturity in the tissue invaded must usually be taken into consideration in the study of the development of older lesions. In the examination of the relation of the growth of the plant to bacterial spread it is soon apparent that growth made during the summer is relatively highly resistant to the bacteria compared with that made during the autumn. An apparent exception to this generalization may be found in badly diseased plants in which the summer growth

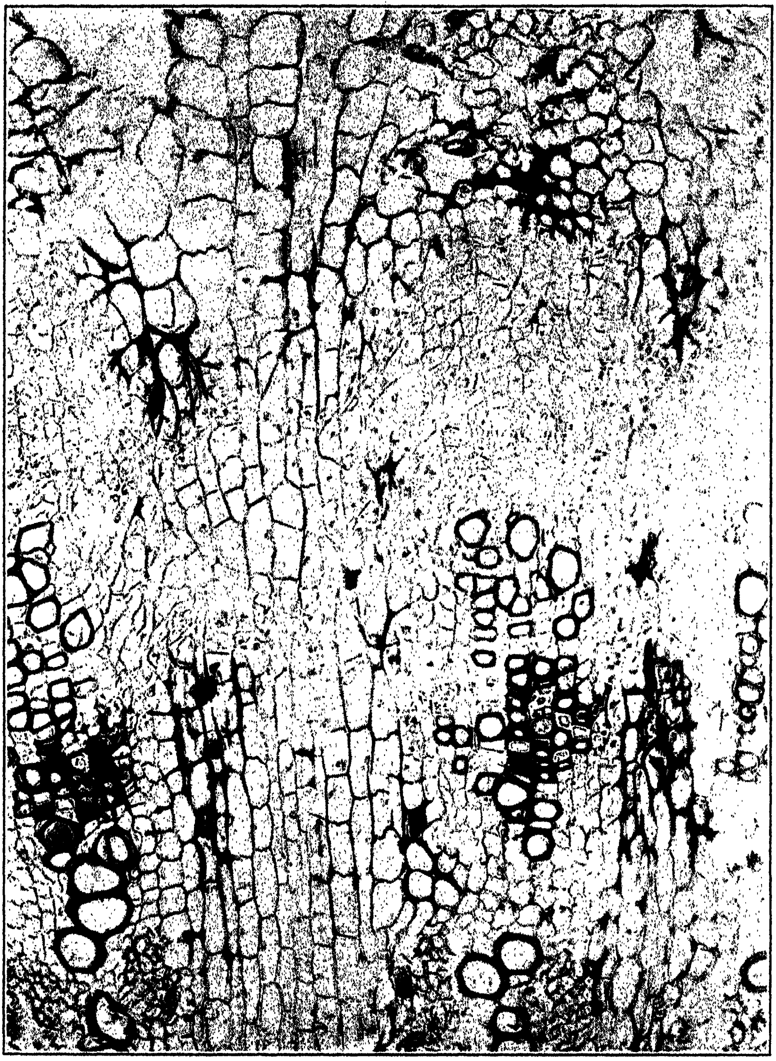

FIG. 4.—Photomicrograph of a stained section of the youngest portion of two vascular bundles in the upper part of the root of a 2-year-old alfalfa plant suffering severe winter injury. The plant was collected at Madison, Wis., May 5, 1927. This root when removed from the ground showed yellow discoloration under the bark very much like that in bacterial wilt, except that it did not extend far below the crown. The section shown here was stained precisely like those stained to reveal the parasitic bacteria. The youngest vessels of large diameter may have been produced in the spring following injury. In some of the small vessels of the autumn growth a gum is found similar in appearance to that in bacterial wilt. The deeply staining material between parenchymatous cells is deposited in and between cells that appear to have been separated mechanically by frost action. Thus winter injury of this character not only simulates bacterial wilt in the discoverable symptoms in the field, but the stained microscopic preparations show a striking superficial similarity to those of plants infected with bacterial wilt. Compare with Figures 2 and 5. × about 200

sometimes resembles that normally made in the autumn. On the whole, however, from the examination of the cross sections of roots it is clearly seen that the spread of the bacteria in autumn wood and perhaps in early spring wood is far greater than that in summer wood. If plants are able to grow vigorously in the summer, the advance of the disease is arrested. In other words, the susceptible tisssue located on either side of the cambium may be described as consisting of hollow cylinders of phloem and xylem connected with each other through the interfascicular cambium. The cylinder of susceptible xylem becomes very thin or perhaps disappears in summer, and reaches a maximum thickness in late autumn and early spring. Curiously enough, these cylinders of tissue susceptible to bacterial invasion are precesely the region in the plant that responds most vigorously to winter injury with the deposit of the material in the cell walls or between the cells. This material stains in a characteristic manner with Gram's stain, as described previously. Sections of roots in which the parasitic bacteria are stained often present a striking similarity in appearance under low magnification to injured but bacterium-free roots stained by the same method. This similarity is shown by a comparison of Figure 4, which is a photomicrograph of a stained section of a root injured by winter freezing, with Figures 2 and 5, which are photomicrographs of similar tissue invaded by the bacteria and stained by the same method.

In the preceding paragraphs consideration has been given only to the development of the bacteria in the parenchymatous tissue of the secondary growth in roots. Secondary growth in the crown is for the most part similar in character to that in roots, and bacterial invasion proceeds in essentially the same manner, though oftentimes apparently more rapidly. The relatively large amount of tissue favorable for invasion and the less compact cellular arrangement in the crown seem to furnish the bacteria favorable conditions for growth. At the bases of the numerous stems and also of the lateral roots the phloem parenchyma is often abundant and seems to be especially favorable for the formation of bacterial pockets. The broken bases of stems are sometimes partly covered with a loosely arranged callus through which the bacteria may become widely distributed. This extensive bacterial invasion of secondary growth in the crown is not often conspicuous because of resulting discoloration or death of tissue. It is important because these bacteria are in a peculiarly favorable situation for invading vascular tissue at an active region of growth where efficient distribution of bacteria through new growth in the taproot below and the primary structure of stems above is assured.

The bacteria may pass up into the innermost vessels of the primary structure of stems at a very early stage in their development and pass from the vessels to parenchymatous tissue even more readily than in roots. Inasmuch as the interfascicular tissue of the outer part of the primary structure and all of the secondary growth is heavily lignified, the bacteria are prevented from passing outwardly as in roots, though they enter the pith readily; and, therefore, in the true stem the bacteria seem to do comparatively little harm, though they may pass almost to its very top. The extent to which stems may be invaded is described more conveniently in a later section.

The basal portions of stems, especially of those produced from the lower part of the crown in autumn, have the morphological character-

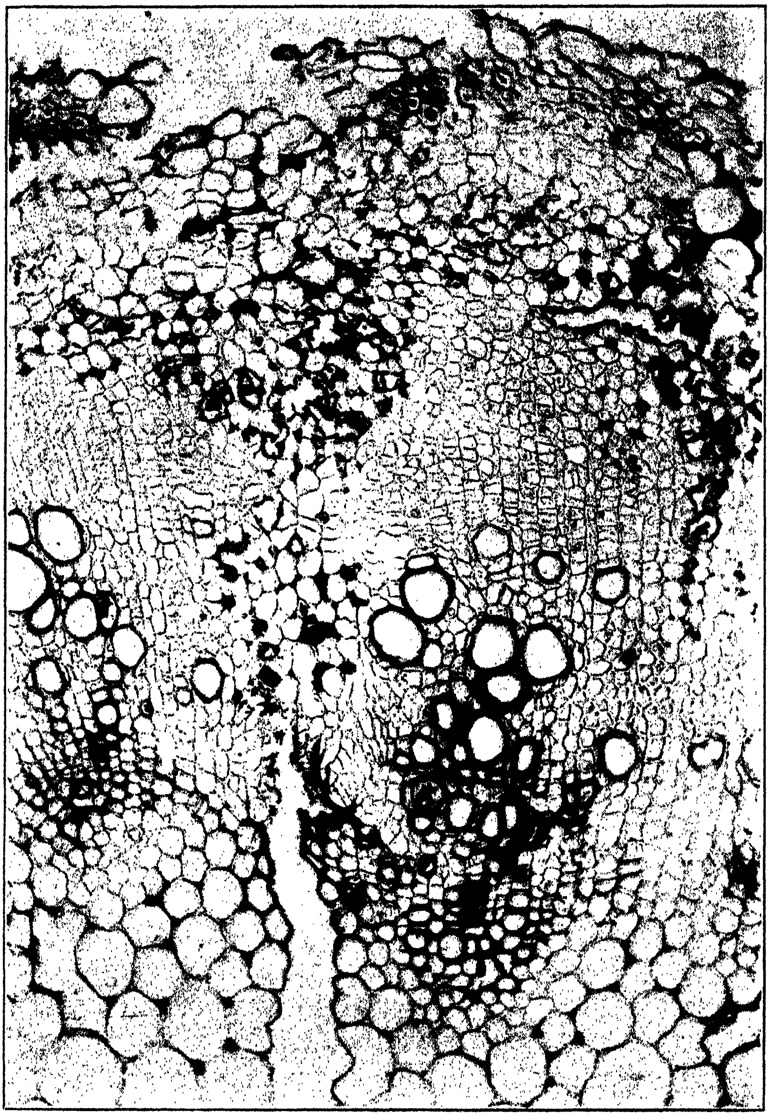

FIG. 5.—Photomicrograph of a stained cross section of a small crown stem or stolon of Semipalatinsk alfalfa, showing two vascular bundles invaded by the wilt-producing bacteria. Infection of the stolon had evidently taken place in the autumn from bacteria advancing through vessels from the invaded root. The inner vessels are plugged and a considerable distribution through parenchymatous tissue had taken place. Freezing injury during the winter destroyed the tissue exterior to the bundle caps shown at the margin of the section, and caused some separation of cells in the pith and the phloem. Some of the large vessels of the wood are probably of spring growth. Although growth at the cambium has begun, note that very little protective cork has formed at the exterior of the wound. At various places in the sections of this stem masses of bacteria were manifestly being released by the disintegration of the outer injured tissue. × about 200

istics of the crown from which they arose. Here the interfascicular tissue is not lignified either in the primary structure or in secondary growth. Therefore, in the crown structure the bacteria pass from the vessels both inwardly through the pith and outwardly as far as the endodermis. The extent to which stem bases of primary crown structure may be invaded in the autumn shoots is shown in Figure 6. Thus the primary structure of the stem bases or crown appears to be a very favorable place in the plant for abundant bacterial develop-

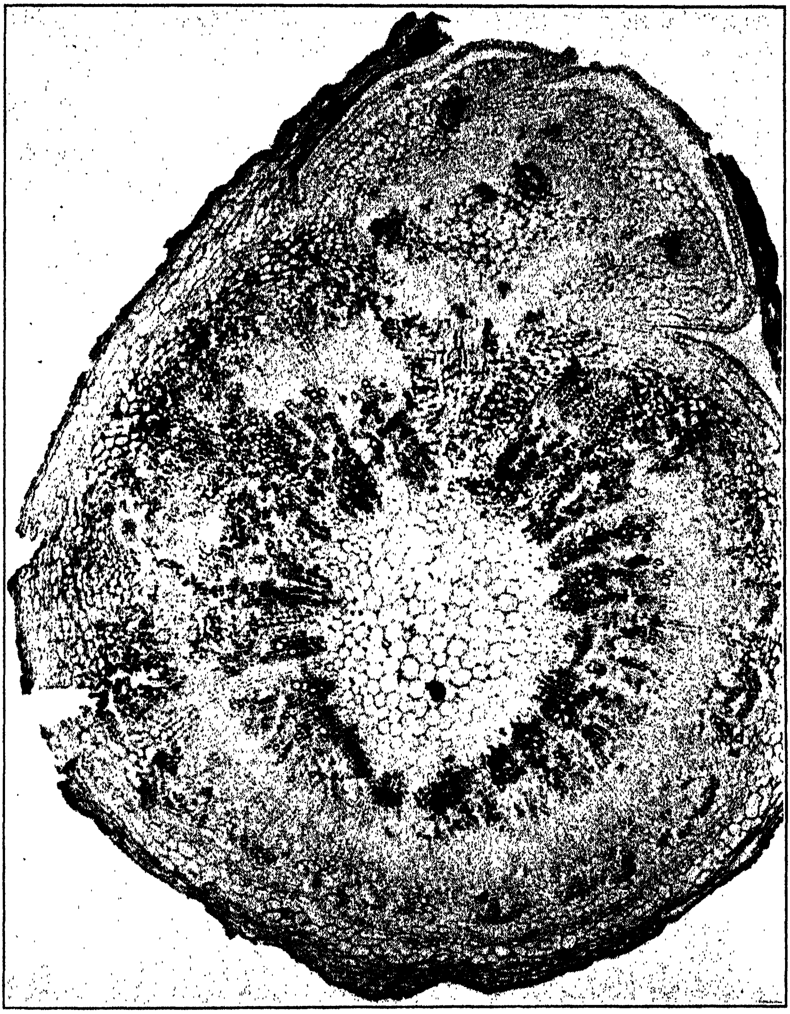

FIG. 6.—Photomicrograph of a stained cross section of the base of a crown shoot of a 2-year-old alfalfa plant badly diseased with bacterial wilt. The plant was collected at Monroe, Wis., November 29, 1927. The extent of bacterial invasion of the tissues at this date is shown by the black masses of bacteria extending all the way from the pith to the cambium on one side of the stem. In general appearance this shoot resembled those shown in Figure 9. Already the primary stem tissue outside the endodermis is cracking open from the diseased condition beneath. The fate of stems like this after heavy freezing is shown in Figure 10. × 40

ment. The significance of this fact will appear in a subsequent discussion of the relation of winter injury to the spread of bacterial wilt.

INVASION OF STEMS IN SEED-BEARING PLANTS

The migration of bacteria up the stems of seed-bearing plants has been examined with a view to determining whether or not the bacteria actually enter the seed. In the autumn of 1925 infected plants bearing seed were collected in Kansas and Idaho, and in 1926 further collections were made in Kansas In both years comparatively few infected plants were found producing seed. With the aid of a microscope many of these were examined in the field for the presence of bacteria in the stems. Comparatively few of the stems showed evidence of the presence of bacteria very far above the crown. Those showing considerable stem invasion were dried and brought to the laboratory for examination. The distance to which the bacteria had advanced in considerable number was determined by pouring agar plates from dilutions or macerated stem fragments from successively higher nodes. When a node was reached at which the bacteria appeared to be very few, fragments at still higher levels were embedded, sectioned, and stained by Gram's method. In this way the bacteria were traced in one plant collected in 1925 to the base of the pedicel bearing a seed pod, and in 1926 the presence of bacteria was demonstrated in the base of a seed pod by staining, but they could not be found near the hilum of the proximal seed in the pod. At the highest point at which the bacteria were found they were not only in vessels but also between parenchymatous cells. In no case were the bacteria found abundantly in the upper parts of the seed plants. Thus seed invasion was not demonstrated, and from the examination of material in these years it seems unlikely that it takes place and certainly that it does not take place abundantly.

EXTENT OF DEVELOPMENT OF BACTERIA IN PLANT ACCOMPANYING APPEARANCE OF SYMPTOMS OF DISEASE IN FOLIAGE AND DEATH OF PLANT

In the preceding description of the invasion of the bacteria through various parts of the plant no attempt has been made to correlate this invasion with the development of the characteristic symptoms that appear in the foliage before the plant dies or to state precisely how the death of the plant is brought about. Inasmuch as most of the plants examined have been sectioned only at one selected portion of the axis for examination of the development of pathological conditions, it is not possible to state with precision the relation of the bacteria to the plant as a whole as the disease progresses. However, it appears that the mechanical obstruction of vessels in the actively conducting wood, together with the cessation of the formation of a new vascular system, accounts largely if not wholly for the dwarfing and death of infected plants. The obstruction appears to be, as a rule, most important in the upper part of the taproot and crown. It is brought about not so much by the bacteria, though they may fill vessels of autumn wood very full, indeed, as by the gumlike material deposited by the plant in vessels in the region of invasion.

In artificial inoculations bacteria may be distributed so widely in the vascular system that they may multiply, plug vessels, and kill the plant before gum is formed. Figure 7 illustrates such a case. But natural infection has not provided instances of this kind.

In old plants growing under conditions where little wood is added each year, the death of the plant may be brought about by the plugging of a comparatively narrow layer of wood. When vascular plugging is sufficiently abundant to retard normal increase in diameter of the root in summer, foliage may begin to wilt on hot days. Up to this time parenchymatous tissue is not usually invaded extensively. When root growth is retarded the new wood contains fewer fibers, and the vessels are usually smaller in diameter and are invaded very promptly. The vascular rays are invaded more extensively at this stage, bacterial pockets may be formed in the slow-maturing young cells near the cambium, and the bacteria pass out through the interfascicular cambium into the phloem. During this stage in development of the disease the shoots produced after cutting are short, with small leaves characteristic of the disease. When the bacteria have entered the phloem around a large part of the circumference of the root near the crown the plant does not survive long even under most

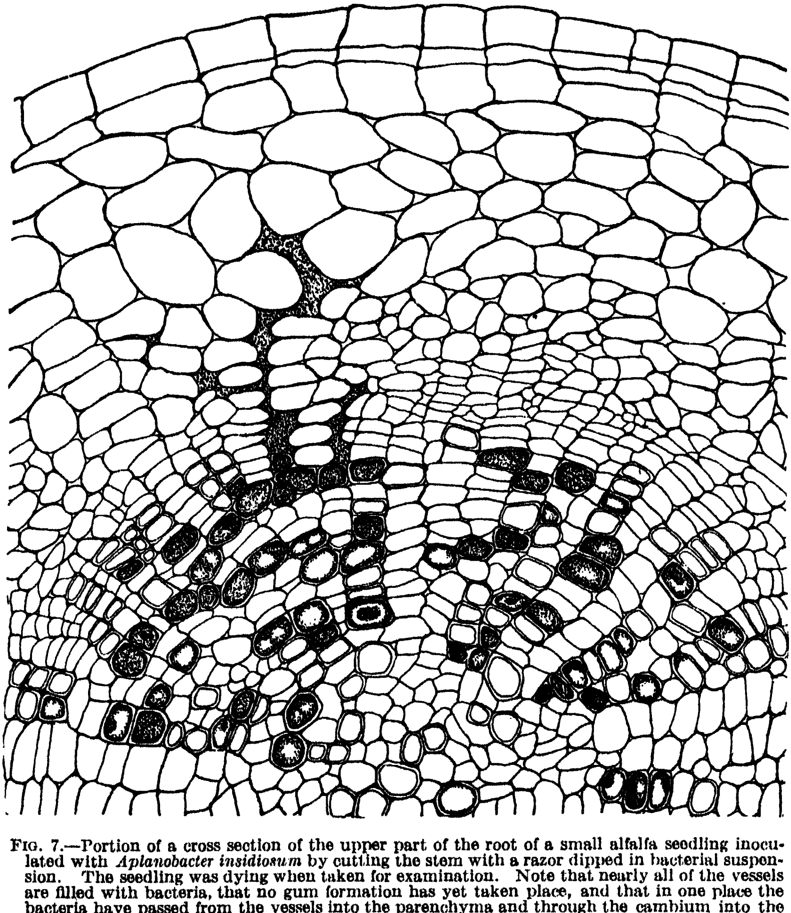

FIG. 7.—Portion of a cross section of the upper part of the root of a small alfalfa seedling inoculated with *Aplanobacter insidiosum* by cutting the stem with a razor dipped in bacterial suspension. The seedling was dying when taken for examination. Note that nearly all of the vessels are filled with bacteria, that no gum formation has yet taken place, and that in one place the bacteria have passed from the vessels into the parenchyma and through the cambium into the phloem. This is the characteristic distribution of the bacteria in the root of small infected seedlings. × 540

favorable external conditions. Before the plant dies the disease may appear to have become parenchymatous in character. Usually the bacteria do not advance into the phloem uniformly around the entire circumference of the root, and the plant may die when they have begun to destroy parenchymatous tissue around less than half of the cambial strands.

LONGEVITY OF WILT-INFECTED PLANTS

EVIDENCE OF LONGEVITY FROM CYTOLOGICAL STUDY

In the earlier part of this paper it has been shown that the first vessels invaded by the bacteria after infection are the outermost ones close beneath the cambium, and that vascular invasion never proceeds inward from this position. Thus it is obvious that however long the plant may live subsequently, these inner invaded vessels will remain as guideposts, marking the time of infection as long as the annual rings of growth can be distinguished. In most plants the summer and autumn layers of each annual ring are readily recognized. Moreover, the parasitic bacteria appear to remain Gram-positive after years of imprisonment in the tissue. Thus from stained sections of roots of infected plants the year in which infection took place can be determined. If the infected plant was growing with moderate vigor, and adding considerable wood each year, it is also possible to determine, approximately, at what time in the year the bacteria entered the vessels. This fact became apparent to the writer during the comparative examination of sections of plants inoculated in the field at various times during two summers at Madison, Wis. Inasmuch as it is a matter of considerable importance to determine at what time plants are infected in the field, the sections of roots of plants inoculated artificially were used as standards of comparison by which the time of infection of diseased plants collected in many fields was estimated. Since the annual growth of most of the field plants was small, it was not often possible to estimate the date of infection closely. The task was simplified, however, by the discovery that in a large majority of the plants the innermost invaded vessels, were in approximately that portion of the annual ring where spring infection was indicated. Evidence of infection in autumn was rare and of doubtful authenticity. Therefore in the estimation of the time of infection of diseased plants those having the innermost invaded vessels in autumn wood were usually regarded as infected in the spring, and those having innermost invaded vessels in late spring or summer wood were designated as infected in the summer.

The oldest record of infection with wilt and subsequent recovery thus far found was obtained in the spring of 1926 from a field said to be 17 years old, located near Abilene, Kans. At the time the collection was made only a few plants showing symptoms of disease could be found; but some of the more vigorous, when dug, disclosed discolorations arranged in a circular manner near the center of the large taproots. When these taproots were sectioned and stained, bacteria were found in the discolored areas. Unfortunately, the annual rings of growth in these roots were so narrow and so poorly differentiated that not all of them could be made out with certainty. However, it appeared from the number of bands of crushed phloem that these plants were at least 15 years old. One root was hollow at the center and had lesions in the cortex, indicating winter injury in the second winter of the plant's growth. The parasitic bacteria were demonstrated in vessels close to the corky layer that surrounded the hollow center, and also in four or five subsequent annual rings, in the last of which they were quite abundant. Beyond this region no evidence of infection was found. In the second root a lesion in the parenchymatous tissue of the wood, which was interpreted as caused by winter injury at the end of the second or third summer's growth, was the innermost point of invasion by the bacteria, which were distributed outwardly through all but the last three annual rings. Thus these two plants appear to contain records of infection by bacterial wilt following winter injury at least 12 years prior to 1926. If this interpretation is accepted, it is clear that the bacterial disease has long been established in this locality, though it has been recognized but recently.

Other instances of recovery and long life of infected plants have not been found. The central wood of the roots of old plants rarely escapes decay as long as 15 years except under conditions of very low rainfall. In fact the plants from Abilene described above were growing in a small tract of sandy soil, and they resembled plants from semiarid districts in having narrow annual rings and little autumn wood.

A large part of the diseased plants from various sources whose roots have been sectioned were showing symptoms of disease when dug and had been infected two or three years previously. Many of them had undoubtedly been infected through injury received in the winter, but in the earlier collections care was taken to select portions of root showing no external injury, and therefore evidence of injury does not appear in many of the sections. For this reason correlations between winter injury and time of infection with wilt can not be satisfactorily made. It is possible, however, in many of the sections to determine approximately the time at which infection took place and the duration of the disease in the plant prior to collection.

The records from 113 plants collected during three years are given in Table 1. From this table it appears that practically three-quarters of the plants collected at random show infection in the spring. In fact, the majority of those indicating infection in the summer are from fields in Kansas, collected after the heavy summer rains of 1927. It may also be inferred that most infected plants live at least one year, and sometimes much longer. This table can not, however, be regarded as an approximate table of life expectancy of infected plants, because of the irregular manner in which the plants were collected.

TABLE 1.—*Time of infection with reference to time of collection of 113 alfalfa plants infected with Aplanobacter insidiosum and collected in 1925–1927*

Year of infection	Number of plants that showed spring infection	Number of plants that showed summer infection
Year collected	25	15
Year previous	38	4
Second year previous	27	3
Third year previous	1	
Total	91	22

LONGEVITY OF PLANTS INOCULATED ARTIFICIALLY

From the preceding discussion it is apparent that the longevity of infected plants may vary considerably in different localities and under different cultural conditions. Experiments have been undertaken to determine the usual length of life of infected plants at Madison, Wis. For the first of these experiments a plot of 3-year-old alfalfa containing strips grown from seeds of the Grimm, Utah, and South Dakota varieties was contributed by F. L. Graber. This was inoculated July 31, 1925, cutting the plants close to the ground with a scythe kept wet with a bacterial suspension. During the rest of that summer only one plant showed symptoms of disease in the foliage. During the following winter, winterkilling damaged the Grimm and nearly destroyed the stands of the other two varieties. Nevertheless the surviving plants grew vigorously. The first cutting of this plot in 1926 was made June 18, and the first symptoms of disease in the surviving plants appeared July 2. By July 15 at least one-half of the surviving plants showed indications of disease, and 165 of these in one corner of the plot were marked with wires for future observation. On September 20, 85 of the marked plants were dead and the majority of the remainder had dwarfed yellow foliage. During the following winter nearly all the plants in the plot were winterkilled and the experiment was discontinued.

This and other experiments designed to test the longevity of infected plants at Madison have been interfered with so badly by winterkilling during the last three years that clear experimental evidence of the usual length of life of infected plants under Wisconsin conditions can not be presented. However, from this experiment and other work now in progress it appears that the usual course of the disease is as follows: Infection takes place chiefly in the spring; few of the infected plants show visible symptoms in the foliage during the following summer, unless winter injury associated with the infection is severe; symptoms begin to appear early in the second summer after infection; and a majority of the infected plants are dead by the end of that summer or are too weak to survive the winter. A small number of the diseased plants live into the third summer after infection, and most of these survivors perish before that summer is past.

WINTER INJURY IN RELATION TO BACTERIAL WILT

WINTER-INJURY LESIONS AS POINTS OF ENTRY FOR BACTERIA CAUSING WILT

The first direct evidence that lesions caused by winter injury serve as foci of infection by the bacteria producing wilt was obtained in the spring of 1927. Among the wilt-infested fields found near Monroe, Wis., in the fall of 1924 was an old stand with a new seeding made that year on lower ground adjoining. The new seeding became infested, and in 1926 a third seeding was made on still lower ground adjacent. The winter of 1926–27 damaged severely both the new seeding and the 3-year-old field. In the latter field many plants were killed and nearly all survivors showed winter injury. Of those remaining in the young stand practically all were likewise damaged in the crown and upper part of the taproot. Heavy showers in the spring of 1927 caused some washing of soil and débris across the two fields.

On June 5, during examination of damaged plants in the youngest field, yellow streaks indicative of wilt infection were found in the wood of many roots. Four of these plants with yellow streaks in the lower part of the root were selected, and the entire upper part of the root and crown was fixed for cytological examination. Sections were made at several selected places in root and crown. In each of these plants winter-injury lesions were found, with abundant bacteria in the surrounding uninjured tissue, the bacterial invasion extending in places all the way to the vessels through which the bacteria had passed both upward and downward, giving rise to the yellow streaks noted in the lower part of the taproot when the plants were collected.

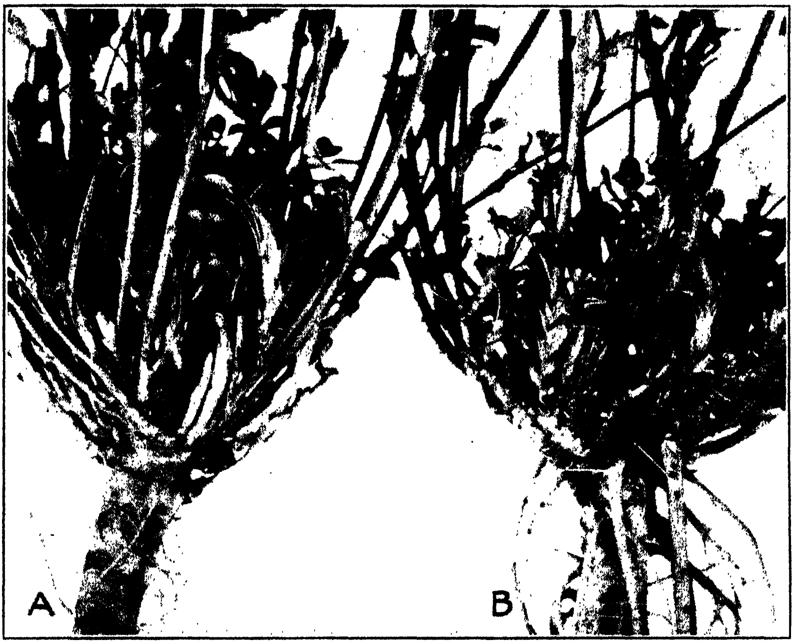

FIG. 8.—Crowns of 4-year-old Grimm alfalfa plants infected with bacterial wilt, found at Monroe, Wis., November 26, 1927, showing the development of young shoots when winter conditions arrived. The taller stems were killed by frost, but the short ones were apparently alive and uninjured at this date. The more severely diseased plant (B) shows small leaves characteristic of the disease. One of the shoots on this crown is shown in Figure 9. × ⅛

The distribution of the bacteria from the winter-injury lesions suggested that the bacteria had entered from them precisely as they had entered wounds in artificial inoculations previously described. The position of the innermost invaded vessels and the comparative absence of gum formation in vessels near those invaded both indicated recent spring infection. In fact, a search of this field as recently as May 5 had failed to detect any diseased plants. The source of the bacteria producing infection was undoubtedly in the older diseased field, in which many plants had been killed and from which the bacteria could easily have been carried in surface water.[11]

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[11] In May, 1928, the infection of plants in a field seeded in 1927 was again traced through wounds caused by winter injury. As in the preceding year, the bacteria could be stained in continuous lines between living cells from the exterior of the wound to the vessels.

RELEASE OF BACTERIA FROM DISEASED PLANTS BY WINTER INJURY

In the autumn of 1927 special attention was given to the development of buds and shoots on both healthy and diseased alfalfa plants, preparatory to a study of the action of freezing upon these shoots during the winter and to the search for new infections the following spring. In the course of this work remarkable development of the bacteria in the bases of shoots formed in the autumn was found. The autumn was unusually warm and moist. The average daily temperature in excess of the average in September, October, and November at Madison, Wis., was 2.4°, 3.5°, and 2.2° F., respectively, while rainfall for the three months was 3.4, 1.81, and 0.62 inches, respectively, in excess of normal. Probably the high temperature and rainfall forced the autumn growth more than usual, though in the absence of precise observation in previous years this can not be determined.

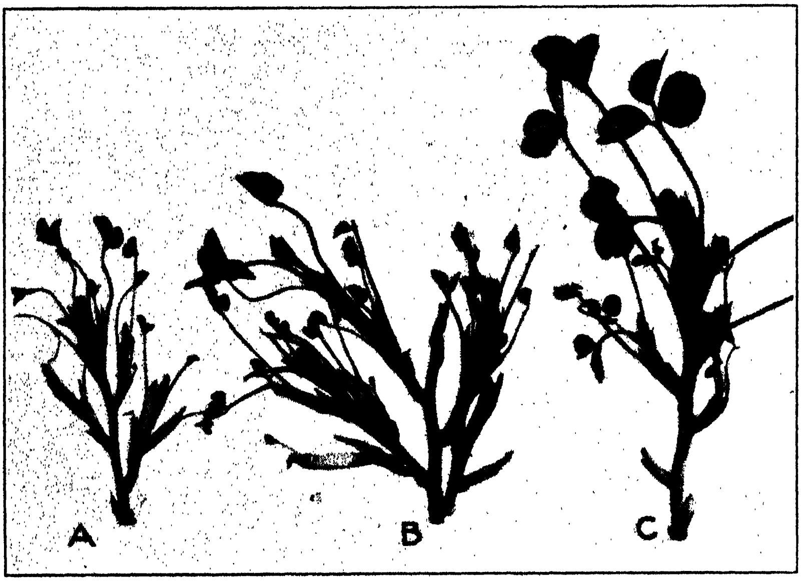

FIG. 9.—Crown shoots of diseased Grimm alfalfa plants (A and B) collected at Monroe, Wis., November 26, 1927, compared with a shoot from a healthy plant (C) in the same field. The central shoot (B) was taken from the plant shown as B in Figure 8. A cross section of the base of the stem of this central shoot was found to be invaded by the parasitic bacteria in a manner very similar to that shown in Figure 6. × 2

The development of shoots from the crowns of 4-year-old diseased Grimm alfalfa plants at Monroe, Wis., on November 27 is shown in Figure 8. These crowns could not be distinguished from those of healthy plants by casual observation. The taller fall stems developed after the last cutting had been killed by frost, but many of the autumn buds had grown out to form a dense green tuft close to the ground. Some of the shoots arising from the bases of these diseased crowns had small leaves characteristic of the disease. One of the shoots from the second plant in Figure 8 is shown in Figure 9. Although clearly showing disease in the foliage, the stem failed to show in a cross section taken at its base more than a very slight water-soaked appearance and a trace of yellow hardly sufficient to distinguish it from a healthy stem; yet a stained cross section of this stem revealed many parasitic bacteria, not only in the vessels, but also in even greater numbers between the parenchymatous cells of phloem and pericycle. The cortex had already broken over the largest bacterial mass, and some of the bacteria may have been released into the soil. After a little experience in searching for these invaded stems at the bases of the crowns of diseased plants they were found in considerable number, though usually only a few were obtained from a single plant. Apparently the autumn stems are similar to autumn wood in the root in possessing a structure through which the bacteria pass readily and in which they may develop in great numbers without immediate destruction of the tissue.

The importance of this abundant development of bacteria in young shoots lies not so much in the damage that can result from the killing of the shoots—though when invaded as badly as that shown in Figure 6 the shoot will not develop far—as in the situation of these bacteria at the surface of the soil in succulent tissue from which they may be easily released and conveyed to other plants. As early as the end of November, before the shoots were injured by frost, the diseased stems were sometimes found cracked to such an extent that a few bacteria must have been released, and it was obvious that winter freezing might serve to release a large part of them. The release of the bacteria by frost action was demonstrated in shoots collected from the same field at Monroe on January 7, 1928. Although green at the top at this time, the bases of the shoots were somewhat soft to the touch, and when sectioned were found to be entirely killed. The parenchymatous tissue was disorganized as shown in Figure 10. The cortex and epidermis were broken and the bacteria, held firmly between the cells when the plant was alive, were now gathered into masses, some of which had already passed out through rifts in the cortex and remained adherent to the exterior. A large part of the bacteria were in a position to be washed out of the collapsed tissue by rain and carried away in the surface water. The bacteria in the frost-shattered stems grew abundantly in culture.

The extent to which the parasitic bacteria are released from diseased plants by frost action and the importance of the bacteria released by this method in the spread of the disease must be determined by future work. Unfortunately, the development of the bacteria in these stem bases and their release is not easily observed without cytological examination. However, the conditions observed at Monroe and described in the preceding paragraph do not appear to be unusual. A similar though less extensive development of bacteria was found in the bases of stems of Grimm and other varieties of alfalfa at Madison, Wis., where the autumn shoots did not appear to develop as far as at Monroe. A similar infestation of autumn stems or stolons of Semipalatinsk alfalfa seems to have taken place at Madison in the preceding autumn. The plants involved in this instance were transplanted roots sent to the writer by N. E. Hansen in the spring of 1926 for the purpose of determining the resistance of this variety to wilt. The roots were set in a field near diseased plants. In the autumn young shoots showed evidence of disease, but the plants were still alive in the spring. A sloughing of the outer bark of the underground stems or stolons indicated winter injury.

The vessels and parenchymatous tissue of all the stems sectioned were found to be invaded by the parasitic bacteria as shown in Figure 5. The position of the bacteria in the innermost vessels indicated that they had entered the shoots in the autumn, and the bacteria in the outermost parenchymatous tissue were clearly being released into the soil following the decay of the tissue killed in the winter. The sloughing of the primary cortex, which is common in the spring, serves to release the bacteria over a longer period of time, and perhaps quite as effectually as the killing of the entire shoot. Thus, in view of what has been learned of the action of frost on the tissue of the alfalfa plant and the evidence cited here that the bacteria are often in a position to be freed from the tissue of diseased plants by this frost action, it appears to the writer that this method of escape is an important stage in the cycle of parasitism of this organism.

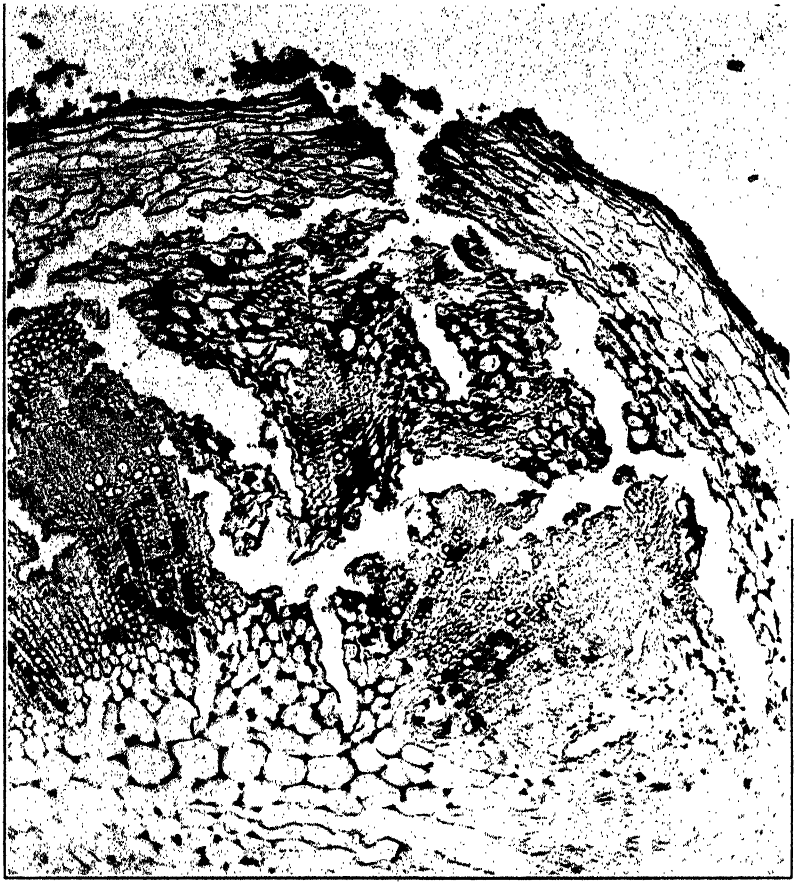

FIG. 10.—Photomicrograph of a portion of a cross section of a crown stem of a 4-year-old Grimm alfalfa plant infected with bacterial wilt, collected January 7, 1928. The plant was taken from the same field from which the crowns shown in Figure 8 were taken. The disorganized condition of the parenchymatous tissue is the result of frost action. The stem is probably entirely killed at this time. The separation of the parenchymatous cells characteristic of freezing injury allows the bacteria formerly held in small masses between the cells to aggregate in larger ones, and the breaking of the outer tissue permits them to be washed out into the surface water. × 100

CYCLE OF PARASITISM OF APLANOBACTER INSIDIOSUM IN RELATION TO THE ALFALFA PLANT

From the evidence assembled in this paper, the life cycle of the bacteria producing wilt appears to be as follows: First, the bacteria develop abundantly in the autumn in all invaded parts of the diseased plants, but especially in the bases of young shoots that are injured or killed by freezing during the winter. Second, the bacteria are released from the stems by the action of frost in separating the cells of the parenchymatous tissue in which the bacteria are held and in breaking the cortex. Third, the bacteria are then distributed in surface water to other alfalfa plants. Fourth, in the spring the bacteria enter cracks in parenchymatous tissue opened by frost or by growth subsequent to frost injury and produce infection. The infected plants produce diseased stems in the following or second following autumn and from them frost action again releases the bacteria in the repetition of the cycle.

The observed behavior of the disease appears to be entirely in accord with this cycle of parasitism. Evidence that spring infection is the rule has already been given. The rapid spread of the disease in infested fields that have suffered severe winter injury has been observed repeatedly. The spread of the disease along the course taken by surface water has also been observed.

It is obvious, however, that other modes of dissemination and invasion contribute to the spread of the disease. Their relative importance remains to be determined and probably varies both locally and from year to year. Distribution of the bacteria by the knife of the mower is undoubtedly not uncommon. Chewing insects are under suspicion as conveyors of the bacteria and as the cause of wounds through which they may enter plants.

Sweet clover is known as another though probably unimportant host for the organism, and still other host plants may yet be found. It may be noted here that the behavior of the bacteria in a few sweet-clover plants has been studied and appears to be substantially the same as in alfalfa. The parenchymatous tissue of fall and early-spring growth of sweet clover is perhaps even more readily invaded than that of alfalfa, while its summer wood is more resistant.

DISCUSSION OF RESULTS

In the study of the development of the bacteria in the tissue of diseased plants as seen in cross sections of roots, the fact that bacteria develop more abundantly both in the parenchymatous tissue and in the vessels of wood produced in the autumn has been interpreted as indicating a high degree of susceptibility of autumn wood to bacterial invasion in comparison with summer wood. The precise character of this susceptibility is not obvious. It seems to be associated with the comparative absence of fibers. It is approximately coextensive with the region that is most susceptible to winter injury in the vascular bundles. It may be due to physical or chemical characteristics of the cell walls. Whatever its nature, it is obvious that if the alfalfa plant did not produce so much autumn wood in the root and crown it might be highly resistant to the disease.

If the sections of roots of the plants from Abilene, Kans., which recovered from the disease, are now examined with this in mind, it is seen at once that the annual rings are very narrow indeed and have very little characteristic autumn wood, so little that the annual growth can not always be made out. The recovery from or endurance of the disease in these plants may have been due to resistance produced by environmental conditions affecting the character of growth, the most conspicuous effect being reduction in the amount of autumn wood. Furthermore, a few collections of roots from dry localities where only one or two crops of hay are cut each year show in most instances very little distinguishable autumn wood; and thus the observed fact that the disease never occurs in such fields except in low moist spots may be due not only to absence of water whereby the bacteria may be distributed, but also to actual resistance of the plants when infected. In further study of the disease the effect of environmental conditions upon the character of growth produced and the susceptibility of that growth to bacterial invasion should be tested.

If the most important cycle of parasitism of the parasitic bacteria proves upon continued investigation to be dependent upon winter injury to furnish wounds through which infection may take place in the manner described here, and if the bacteria do not live long in the soil or in other hosts a satisfactory control of the disease can undoubtedly be achieved by preventing the washing of the bacteria from old diseased fields to new seedings. This may involve the destruction of old stands in which the disease is abundant, when these are so located that surface water passing across them floods other fields. Old alfalfa fields when plowed up should not be reseeded until all plants that may carry the disease have been destroyed. Even when these precautions are taken, it appears that the disease may enter new fields located in infested localities, but not as early or as abundantly as when water can convey the bacteria readily from diseased plants.

In the absence of resistance in present varieties, and with uncertain success by the use of disease-escaping hardy varieties, a critical examination of the epidemiology of the disease is needed to define more precisely the cultural practices that may eliminate or reduce the disease in the several widely different agricultural districts where it has become an important enemy of the alfalfa crop.

SUMMARY

In the course of the study of bacterial wilt of alfalfa, observation of the disease in the field has suggested that winter injury of alfalfa plants provides wounds through which the bacteria often enter the plant. Following this suggestion, the relation of the bacteria to the host plant has been examined, and in this paper this relation and the effect of freezing injury upon the development of the disease are described.

The yellow discoloration of the wood of the root characteristic of the disease is due partly to a yellow insoluble material in the vessels occupied by or in the vicinity of the bacteria and partly to a relatively soluble stain that may diffuse for some distance from the infected region. Therefore the bacteria are usually not so widely distributed as the stain. In stems the stain does not develop so abundantly as in roots.

The gum formed in vessels near the bacteria appears to be a product of the plant, not of the bacteria.

The bacteria pass more readily from wounds in the phloem between the cells of the phloem rays, ray cambium, wood rays, and wood parenchyma into vessels.

In the vessels the bacteria are carried by water long distances vertically through open passages, and some open communication in a tangential direction permits to some degree distribution around the circumference of the root. The bacteria seem to advance through successive layers of wood by invading young parenchymatous tissues of the crown and the upper part of the root.

Parenchymatous cells do not permit intracellular invasion until they are nearly fully expanded though they are sometimes broken down with the formation of bacterial pockets. Parenchymatous cells of autumn and perhaps of early-spring growth admit of extensive intercellular invasion in autumn and spring but appear to become resistant in summer. Summer tissue, especially of the wood, appears to be relatively resistant to invasion at all times.

Seed infection has not been demonstrated, although the bacteria may be found far up the stems of seed-bearing plants and in one plant were identified in the base of a seed pod.

The symptoms of disease in the foliage of plants are associated with a considerable plugging of the vessels in the zone of wood that is active in conducting water, and in very young plants death sometimes appears to follow from such plugging. In plants of several years of age in the field a considerable invasion of parenchymatous tissue about the fascicular cambium of some of the bundles and a cessation of growth from the cambium usually precede death.

From cytological examination of roots of diseased plants collected in many fields it appears that at least 75 per cent of the plants examined thus far were infected in the spring. Although the longevity of infected plants varies greatly, it appears that the greater number show conspicuous symptoms of disease and die in the second year after infection.

Evidence is presented indicating that the prevalence of spring infection referred to previously is due to the entrance of the bacteria through wounds caused by winter injury.

An important source of the bacteria causing spring infection may be found in old diseased plants where the organism has developed abundantly in the bases of shoots produced in the autumn. Freezing injury or winterkilling of these shoots releases the bacteria into the surface water that may carry them to other plants.

If the cycle of parasitism outlined here is the most important method whereby the bacteria are distributed in epidemics of the disease, it appears that bacterial wilt may be greatly delayed or avoided by placing new seedings where they are inaccessible to surface wash or flooding by water that has passed among diseased plants.

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NEMATODES INHABITING THE CYSTS OF THE SUGAR-BEET NEMATODE (HETERODERA SCHACHTII SCHMIDT)[1]

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INTRODUCTION

While examining the 38,128 cysts of *Heterodera schachtii* Schmidt used in studying its dormancy,[2] the writer found many cysts that contained other species of nema. In certain fields these species occurred so frequently that the possibility was suggested that they were feeding upon the eggs and larvae contained in the cysts.

METHODS

These cyst-inhabiting species were present in both fields where mononchs[3] were studied, and it was possible to secure data for both projects from the same series of soil samples. These samples were collected by the Cobb method,[4] in tubes having an area of one-millionth of an acre. Each sample examined was an aliquot part of two to six samples, usually two. The soil of each 2-inch depth was collected separately, care being exercised not to mix it with soil from above or below the specified depth. The samples were washed by the Cobb sifting and gravity method, and a determination was made of the nema present, both within the cysts and living free in the soil.

RESULTS OF SOIL EXAMINATIONS

The 1923 data contained in Tables 1, 2, and 3 for the field at Salem, Utah, are typical of those secured and, being the most complete, are presented here as representative of the generally existing conditions. Two species, *Cephalobus oxyuroides* de Man 1884 and *Acrobeles bütschlii* (*Acrobeloides*) (de Man) 1885 (fig. 1), predominated in the cysts.

Table 1 gives the number and distribution of the collected cysts of *Heterodera schachtii*. Of these 4,997 cysts examined, 1,021 contained an estimated total of 103,602 eggs and larvae. The remaining 3,976 were empty, the eggs having hatched and the larvae escaped into the soil. These empty cysts represent an accumulation of many years, for they are of a cutinous, almost indestructible material, and great numbers accumulate in the infested soil.

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[1] Received for publication July 31, 1928; issued December, 1928.

[2] THORNE, G. LENGTH OF THE DORMANCY PERIOD OF THE SUGAR-BEET NEMATODE IN UTAH. U. S. Dept. Agr. Circ. 262, 5 p. 1923.

[3] THORNE, G. THE LIFE HISTORY, HABITS, AND ECONOMIC IMPORTANCE OF SOME MONONCHS. Jour. Agr. Research 34: 265-286, illus. 1927.

[4] COBB, N. A. ESTIMATING THE NEMA POPULATION OF SOIL, WITH SPECIAL REFERENCE TO THE SUGAR-BEET AND ROOT-GALL NEMAS, HETERODERA SCHACHTII SCHMIDT AND HETERODERA RADICICOLA (GREEF) MÜLLER, AND WITH A DESCRIPTION OF TYLENCHOLAIMUS AEQUALIS N. SP. U. S. Dept. Agr., Bur. Plant Indus., Off. Agr. Tech. Circ. 1, 48 p., illus. 1918.

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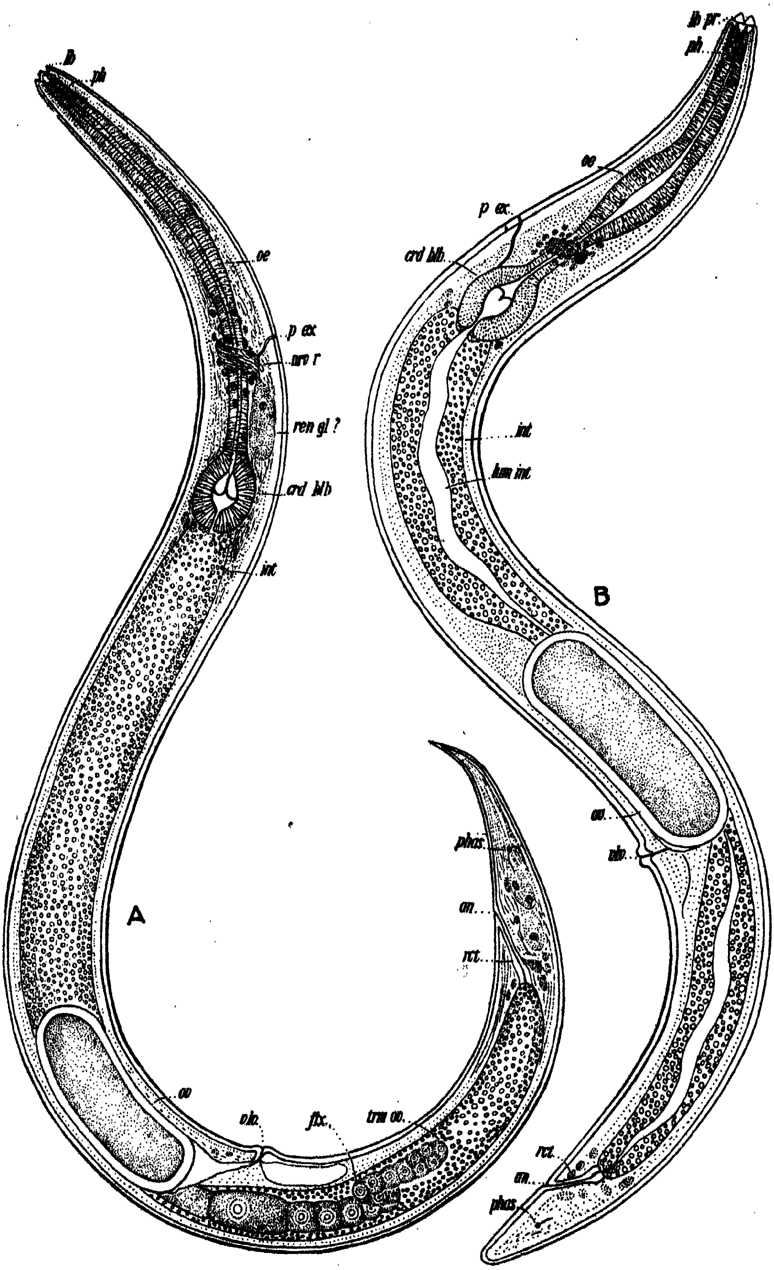

FIG. 1.—Adults of *Cephalobus oxyuroides* (A) and *Acrobeles bütschlii* (*Acrobeloides*) (B). × 450

Key: *an*, anus; *crd blb*, cardiac bulb; *flx*, flexure of ovary; *int*, intestine; *lb*, lip or labial projection; *lum*, lumen; *nrv r*, nerve ring; *oe*, esophagus; *ov*, ovum; *p ex*, excretory pore; *ph*, pharynx; *phas*, phasmids; *pr*, probolae; *rct*, rectum; *ren gl(?)*, renette gland; *trm*, terminus; *vlv*, vulva.

TABLE 1.—*Number and distribution of cysts of Heterodera schachtii at Salem, Utah, in 1923*

Depth in the soil	Date collected							
	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29[a]	Sept. 5	Nov. 6
Inches	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
1-2	128	36	389	34	27		50	131
3-4	61	58	240	80	57		133	146
5-6	58	27	162	113	35		210	144
7-8	16	17	100	35	82		112	107
9-10	88	20	78	139	121		153	223
11-12	68	46	40	37	56		74	167
13-14	17	52	7	9	22		87	219
15-16	3	36		4			69	208
17-18							41	49
19-20	4	4					25	23
21-22							11	9
23-24								
Total[b]	443	296	1,016	451	400		965	1,426

[a] Sample taken outside area infested by *Heterodera schachtii.*
[b] Grand total, 4,997; average population per acre, 624,375,000.

Table 2 shows the distribution of the 386 *Cephalobus oxyuroides* that were found in the samples. None of these was found living free in the soil. They were secured from 201 cysts, 70 of which contained eggs or larvae of *Heterodera schachtii*, and 131 were empty.

A comparison of Tables 1 and 2 shows two interesting facts: (1) On August 29 the sample was taken outside the area infested by *Heterodera schachtii*, and on that date no *Cephalobus oxyuroides* were collected; (2) in the samples collected September 5 and November 6, *H. schachtii* was found to a depth of 22 inches. On these same dates the many specimens of *C. oxyuroides* were found deep in the soil, indicating that at this season of the year they selected that point of habitat if their preferred food was present.

TABLE 2.—*Number and distribution of Cephalobus oxyuroides at Salem, Utah, in 1923*

Depth in the soil	Date collected							
	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29[a]	Sept. 5	Nov. 6
(*Inches*)	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
1-2				6				
3-4								
5-6			2					
7-8	2	2	8				4	
9-10		10	5		1			
11-12	12	41	9		10		17	
13-14	17	3	14		1			35
15-16	19	2					52	26
17-18							6	40
19-20							4	9
21-22							18	11
23-24								
Total[b]	50	58	38	6	12		101	121

[a] Sample taken outside area infested by *Heterodera schachtii.*
[b] Grand total, 386; average population per acre, 48,250,000.

Table 3 gives the distribution of 504 *Acrobeles* (*Acrobeloides*) *bütschlii* found in the samples. Of these, 384 were secured from 317 cysts and 120 were from the soil. Of the cysts inhabited by this species, 134 contained eggs or larvae and 183 were empty. The numbers occurring in a cyst varied from 1 to 11. Frequently an adult female was present with a number of progeny varying in size from those just hatched to some as large as the mother. This species is more generally distributed throughout the beet-growing regions of the Western States than *Cephalobus oxyuroides* and very frequently is found living free in the soil where *Heterodera schachtii* is not present. In this connection it will be noted that the sample collected August 29 contained 45 *A. bütschlii*, although *H. schachtii* was not present in that portion of the field. In many fields heavily infested with *H. schachtii* it is not unusual to find hundreds of *A. bütschlii* living free in the soil with rarely an individual present in the numerous cysts, indicating that preferred food is abundant outside the cysts.

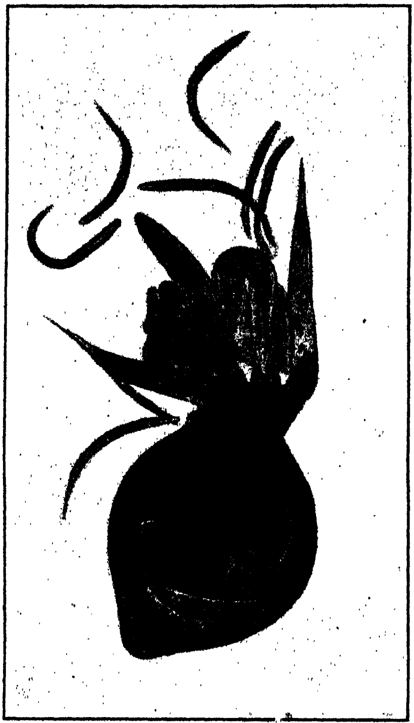

FIG. 2.—Photomicrograph of female *Heterodera schachtii* containing adults and young of *Cephalobus elongatus*. Apparently this was an unfertilized female and the Cephalobi had entered the body after its death; it was therefore not a case of true parasitism. × 70

TABLE 3.—*Number and distribution of Acrobeles (Acrobeloides) bütschlii at Salem, Utah, in 1923*

Depth in the soil	Date collected							
	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29[a]	Sept. 5	Nov. 6
(*Inches*)	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
1-2	4			6				5
3-4			17	2		1		1
5-6	20	2	18	1		4		
7-8	8	4	47				5	
9-10	8		105	3	3	4		
11-12	73	3	40		7	36	3	
13-14	2	5	18		14		1	3
15-16		1					12	9
17-18				1			1	4
19-20							2	
21-22							1	
23-24								
Total[b]	115	15	245	13	24	45	25	22

[a] Sample taken outside area infested by *Heterodera schachtii*.

[b] Total number of cysts, 384; total number of free living, 120; grand total, 504; average population per acre, 63,000,000.

Other species of nematodes found in small numbers in the cysts of *Heterodera schachtii* from fields located in various parts of Utah, Idaho, Colorado, and California are as follows: *Acrobeles ankyrus* Thorne, *A. complexus* Thorne, *A. contractus* Thorne, *A. ctenocephalus* Thorne, *A. minimus* Thorne, *A. symmetricus* Thorne, *Cephalobus elongatus* de Man (fig. 2), *C. persegnis* Bastian, *C. striatus* Bastian, *Dorylaimus obtusicaudatus* Bast. (fig. 3), *Tylenchus* sp., and *Plectus* sp.

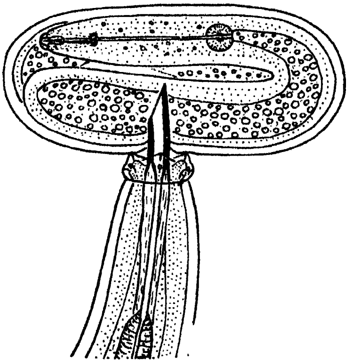

FIG. 3.—Sketch of the head of a young *Dorylaimus obtusicaudatus* with an egg of *Heterodera schachtii* impaled upon the spear. The young of this species are quite frequently found inhabiting the cysts of *H. schachtii*, but only three specimens have been seen with eggs impaled upon the spear. This species is not generally predacious, and these occurrences are probably accidental. × 350

ECONOMIC IMPORTANCE

Observations on the habits of many species of Acrobeles[5] and Cephalobus have led the writer to believe that primarily they are saprozoans. From the fact that eggs and larvae were present in only 41 per cent of the cysts inhabited by *Cephalobus oxyuroides* and *Acrobeles bütschlii*, it seems evident that they must have been feeding on the remnants of the internal organs of the female *Heterodera schachtii* and not on the eggs and larvae. Both of these species are too small to swallow an egg entire, and neither possesses a spear or teeth with which the eggs could be broken. Even if they were able to attack and devour the egg and larvae, the small percentage destroyed would cause no appreciable diminution in the population of *H. schachtii.* It will thus be apparent that these examinations fail to indicate that the nemas observed in Heterodera cysts are of economic importance in the control of this highly important sugar-beet pest.

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[5] THORNE, G. THE GENUS ACROBELES VON LINSTOW, 1877. Amer. Micros. Soc. Trans. 44: 171-210 illus. 1925.

SEED-COAT STRUCTURE AND INHERITANCE OF SEED COLOR IN SORGHUMS[1]

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INTRODUCTION

Seed color is one of the most definite taxonomic characters of the sorghum plant, and it is also of considerable economic importance because of its influence on the market value of the grain. The mode of inheritance of kernel color, however, has been perplexing, and various Mendelian ratios have been obtained by plant breeders who have studied crosses of different sorghum varieties. Most of these genetic data have been obtained on the basis of surface examination and without considering the structure and pigmentation of the various layers of the seed coat. A genetic study of crosses between Red Amber and Freed sorgos and Standard feterita and their reciprocals is presented here. The interpretation of these is based on the microscopic study of the seed coats of the segregates and of several standard varieties of sorghum.

LITERATURE REVIEW

Graham[3] of India in 1916 classified the segregates from a number of crosses of juar (sorghum) and found in one cross plants having three seed colors distinguished as red (*RRYY*), yellow (*rrYY*), and white (*RRyy* or *rryy*). Taking the families in which all three colors occurred, he found a total of 340 red-seeded, 96 yellow-seeded, and 139 white-seeded plants, or a close approximation to a 9:3:4 ratio. He also found on crossing a number of whites with one pure yellow that the whites proved to be of at least two genotypes. One white-seeded plant crossed with yellow produced red-grained F_1 plants and another produced yellow-grained ones. This led to the assumption that some of the whites contained one of the necessary factors for red, which in the presence of the yellow factor led to the production of red color. Graham made no statement as to the location of pigments in the varieties under observation.

Vinall and Cron[4] studied a cross of feterita × Red Amber sorgo and its reciprocal, grown at Chillicothe, Tex., and obtained a ratio of 15 plants with pigmented seed to 1 with white seed. They as-

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[1] Received for publication Aug. 24, 1928; issued December, 1928. Certain phases of the study here reported were submitted in June, 1923, to the faculty of the graduate school of the University of Minnesota in partial fulfillment of the requirements for the degree of master of science. These have been supplemented by additional data.

[2] The writer takes this opportunity to acknowledge the aid of Dr. H. K. Hayes, professor of plant breeding, University of Minnesota, for valuable criticism and advice. Dr. J. H. Parker, of the Kansas State Agricultural College, has generously permitted the writer to use data collected jointly. Prof. W. E. Davis, of the Kansas Agricultural College, assisted materially in developing the microscopic technic necessary for studying the seed coats of sorghums.

[3] GRAHAM, R. J. D. POLLINATION AND CROSS-FERTILIZATION IN THE JUAR PLANT (ANDROPOGON SORGHUM, BROT.). India Dept. Agr. Mem., Bot. Ser. 8: [201]-215, illus. 1916.

[4] VINALL, H. N., and CRON, A. B. IMPROVEMENT OF SORGHUMS BY HYBRIDIZATION. Jour. Heredity 12: 435-[443], illus. 1921.

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sumed the factors for color to be *R* (red) and *B* (brown), either one of which alone or in combination produced color, while the recessive condition for both *R* and *B* resulted in the production of white seed.

Sieglinger[5] in the cross Sunrise kafir × feterita and its reciprocal obtained a dihybrid ratio of 9 plants with brown seeds and brown nucellar layer, 3 with chalky-white seeds and brown nucellar layer, and 4 with glossy-white seeds and no brown nucellar layer. To explain this inheritance Sieglinger assumed the following factors:

B, a factor for brown nucellar layer which also may cause brown in the epidermis if *S* is present. Its allelomorph *b*, gives kernels without a brown nucellar layer.

S, a factor for smooth or glossy pericarp. When *S* is present the pericarp is glossy and may be creamy white, as in white kafir, or may carry other colors. Its allelomorph *s*, gives a chalky-white pericarp. Brown does not appear in the pericarp of an *ss* plant.

The results expected in the F_2 generation, from an independent recombination of the two factors just designated, would produce three phenotypes, classified as follows:

Brown seeded, with brown nucellar layer	Chalky white seeded, with brown nucellar layer	Glossy white seeded, with no brown nucellar layer
1 *BBSS* 2 *BBSs* 2 *BbSS* 4 *BbSs*	1 *BBss* 2 *Bbss*	1 *bbSS* 2 *bbSs* 1 *bbss* [a]
9	3	4

[a] Sieglinger recognized that this phenotype had a chalky-white seed coat, but he was not able to distinguish it from the other individuals of this group because of the discoloration of this seed from weathering.

By testing the progeny of F_2 heads in the F_3 generation, Sieglinger[6] was able to substantiate the above factorial constitution. He obtained like results in the cross Sunrise kafir × Blackhull kaoliang and assumed that the latter variety had the same seed characteristics as feterita. In the cross Sunrise kafir × Red kafir, Sieglinger obtained a ratio of three plants with red seed to one plant with white seed. No nucellar layer with pigmentation was present in either the Sunrise or Red kafir parents, so that the pericarp color differed by only one factor. The factors influencing color in the Sunrise and Red kafir cross were designated as *rr* for Sunrise kafir and *RR* for Red kafir. The *S* factor supposedly is present in both parents, and the red color of the F_1 was considered due to the combination *Rr*. Segregation in the F_2 generation gave the monohybrid ratio of three red to one white. A similar result was obtained from the cross White kafir × Red kafir. As would be expected in the cross between the two white-seeded varieties, White kafir and Sunrise kafir, no plants with colored seed were obtained.

Sieglinger[6] also determined the inheritance of three color factors in the cross Standard feterita × Red kafir. As noted above, Red kafir differs from Sunrise kafir in that it has a colored pericarp, both lacking the nucellar layer with its accompanying pigmentation. Sieglinger designated the genetic color make-up of feterita as *BBssrr*, and that for Red kafir as *bbSSRR*. The effects of the *Bb*, *Ss*, and *Rr* factors were the same as in the previously noted crosses, the presence of either the *S* or the *R* factor causing the brown color to appear in the epidermis of the seed. On a basis of three color factors the segregation in the F_2 generation should give 45 plants with brown seeds and brown nucellar layer, 3 with white seeds and brown nucellar layer, 12 with red or light-red seeds and no brown nucellar layer, and 4 with white seeds and without a brown nucellar layer. The observed ratios were in close agreement with this calculated distribution.

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[5] SIEGLINGER, J. B. SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES. Jour. Agr. Research 27: 53-64. 1924.

[6] SIEGLINGER, J. B. Op. cit.

Conner and Karper[8] obtained a ratio of three plants with colored grain to one with white in a cross between Dwarf White milo and Dwarf Yellow milo. These investigators also obtained a segregation of 1 plant with red kernels, 2 with pale-red kernels, and 1 with white kernels in a cross between Blackhull White kafir and Red kafir, or a ratio of 3 colored to 1 white. Similar results were obtained from a cross of Blackhull White kafir × Pink kafir, except that the colored seed was designated as "pink" and "pale pink" in the F_2 generation. Representative types of homozygous pink, heterozygous pink, and homozygous white from the F_2 generation were grown in the F_3 generation and the correctness of the 1 : 2 : 1 segregation was substantiated. The F_4 progeny from 72 heterozygous individuals of the F_3 generation did not, however, give a very close approximation to the expected 3 : 1 ratio, the observed numbers being 3,163 colored and 1,477 white. The authors state: "This is evidently a poor fit for the classes, for reasons not known at this time, except for lack of proper determination of classes."

From the above review it is apparent that the inheritance of grain color in sorghums is complex and has not been worked out definitely. The writer believes, however, that a knowledge of the structure of the seed coat and the location of pigmentation offers a correct basis for interpreting data on the inheritance of seed color. The writer proposes some slight modifications of Sieglinger's hypothesis as to the effects of the several factors influencing the inheritance of color. It is believed that the modification, which is based on studies of seed structure, is substantiated both by the data of others just cited and by additional data presented in this paper.

INVESTIGATIONS

SEED COATS OF SORGHUMS

The sorghum kernel is a caryopsis. The pericarp, derived from the wall of the ovary, consists of epidermis, hypoderm, mesocarp, and cross and tube cells. Winton[9] found that the grains of some varieties of sorghum possess a nucellar layer, while in other varieties this layer is absent. The writer's observations agree with this conclusion. The nucellar layer is sometimes referred to as the hyaline layer. Morphologically it is derived from the nucellus. In some varieties of sorghum the developing endosperm completely absorbs the nucellus, while in others a layer of cells derived from the nucellus remains. Immediately under the nucellar layer is the aleurone layer, a part of the endosperm.

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[8] CONNER, A. B., and KARPER, R. E. THE INHERITANCE OF SEED COAT COLOR IN CERTAIN CROSSES IN GRAIN SORGHUM. Jour. Amer. Soc. Agron. 15: 338-344. 1923.

[9] WINTON, A. L. THE ANATOMY OF THE FRUIT OF CERTAIN CULTIVATED SORGHUMS. Conn. State Agr. Expt. Sta. Ann. Rpt. (1902) 26: 326-338, illus. 1903.

The color relationships of the different structures of the seed coat in the sorghum varieties studied by the writer were very definite. The nucellar layer when present contained a reddish brown pigment in all of the varieties studied. Color due to this pigmentation will be designated nucellar color.

The mesocarp is a starchy structure. When it is thick it obscures the color of the nucellar layer. If it is vestigial in development, the pigment in the nucellar cells is visible in varying degrees of intensity through the more or less corneous epidermal and hypodermal cells of the pericarp. Careful examination revealed no definite areas of pigmentation in the mesocarp itself. In a very few specimens small irregular areas appeared slightly colored as if the pigment had diffused from the nucellar layer into the adjacent starchy cells of the mesocarp. These colored mesocarp cells did not appear as compactly arranged as others in this structure. From this study it is at least safe to conclude that the starchy cells of the mesocarp carry pigments but rarely, and then only in very small irregular areas. In the seeds of two crosses of Pink kafir × feterita the mesocarp was found to be entirely missing, although the nucellar layer was present.[10]

The epidermal and the hypodermal cells of the pericarp are the carriers of pigmentation when color is found in the outer portion of the seed coat. In this paper such coloration is termed "pericarp color." Whether this pigment is of the same origin as that found in the nucellar layer was not determined by the writer. An intensely colored pericarp seems to be associated with a vestigial mesocarp and a pigmented nucellar layer.

The seed-coat structures of five varieties of sorghum are shown diagrammatically in Figure 1. Feterita has a chalky-white seed. It has a well-developed nucellar layer, but the color is obscured by the thick, highly developed mesocarp. The pericarp is colorless. Red Amber sorgo has a well-developed nucellar layer, a thin mesocarp (about 10 μ in thickness), and a reddish brown pericarp. Blackhull kafir has a colorless pericarp and no nucellar layer except for slight traces that are believed to be responsible for the colored specks in the seed coat. Yellow milo lacks the nucellar layer and has a poorly developed mesocarp. It has a salmon-yellow pericarp. Both Freed sorgo and White milo have poorly developed mesocarps and lack nucellar layers. Both lack percarp color.

RELATION OF SEED-COAT STRUCTURE TO GENETIC FACTORS

The relation of the seed-coat structures to the genetic factors that control color inheritance is assumed by the writer to be as follows:

The factor *B* determines the presence of the pigmented mucellar layer and also is considered responsible for the development of the traces of nucellar color that may pass into the mesocarp or even into the pericarp if the mesocarp is poorly developed. *S* is a dominant factor, the presence of which causes a vestigial or poorly developed mesocarp. The recessive allelomorph *s* determines a well-developed, starchy, opaque mesocarp, the presence of which inhibits even a slight expression of nucellar coloration, due to *B*, in the epidermal and hypodermal cells. When *R* is present, color is produced in the epidermal and hypodermal cells. When *B* and *R* are both present together with *S*, the factor for a poorly developed mesocarp, the effect is an intense coloration.

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[10] The seeds of these crosses were supplied by J. B. Sieglinger, Office of Cereal Crops and Diseases, and R. E. Getty, Office of Forage Crops, Bureau of Plant Industry, U. S. Department of Agriculture.

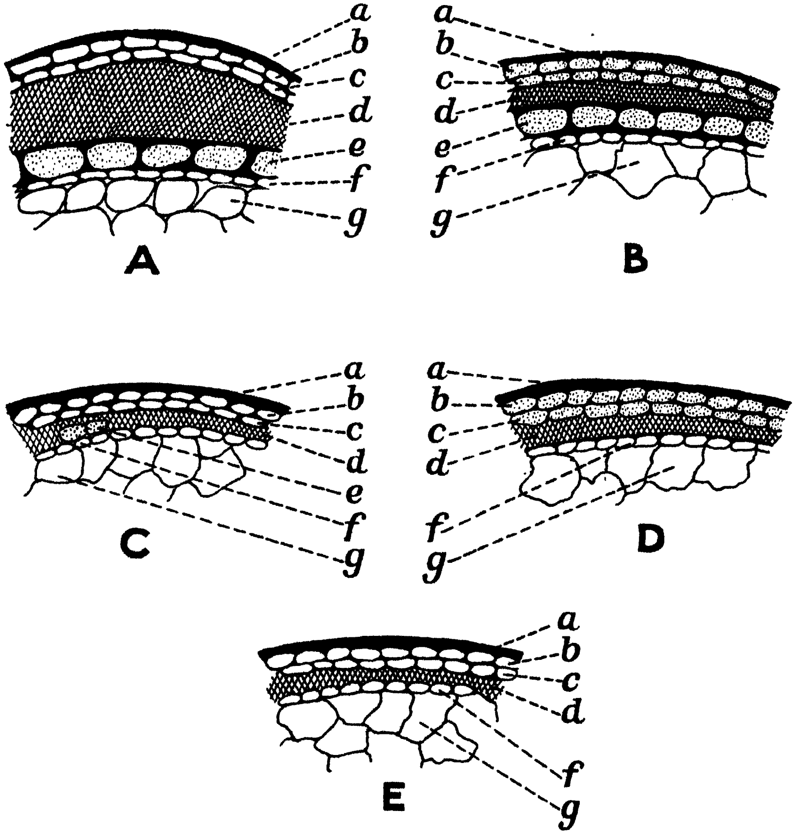

Fig. 1.—Diagrammatic sections of the seed coats of five sorghum varieties: *a*, Cuticle; *b*, epidermis; *c*, hypoderm; *d*, mesocarp; *e*, nucellar layer; *f*, aleurone layer; *g*, endosperm. A, Feterita; colorless epidermis and hypoderm, highly developed mesocarp, and nucellar layer; B, Red Amber; colored epidermis and hypoderm, thin mesocarp, nucellar layer; C, Blackhull kafir; colorless epidermis and hypoderm, thin mesocarp, and nucellar layer absent except for slight remnants; D, Yellow milo; colored epidermis and hypoderm, thin mesocarp, nucellar layer absent; E, Freed sorgo and White milo; colorless epidermis and hypoderm, thin mesocarp, nucellar layer absent

It is assumed that there are two classes of white seed, namely, pseudowhite and true white. The pseudowhites (*BBssrr* and *Bbssrr*) are so designated since the nucellar color factor *B* is present while the pericarp color factor *R* is absent. When *B* is present there is color in the nucellar layer, but it is masked by the well-developed mesocarp due to *s*. The true whites have the factorial constitution *bbssrr*, *bbSsrr*, or *bbSSrr* and are true whites because of the absence of both the pigmented nucellar layer and pericarp color.

The pseudowhites can usually be distinguished by removing the pericarp with a penknife to ascertain whether or not the colored nucellar layer is present.

Because of the nucellar layer, varieties of the pseudowhite group when crossed with other varieties may give coloration in progenies, depending on relative mesocarp thickness. When two varieties of true-white factorial constitution are crossed, only white-seeded progenies can be obtained. The pericarp of sorghums of the factorial constitution *BBSSrr*, *BBSsrr*, *BbSSrr*, and *BbSsrr* may possess some slight degree of coloration even though the pericarp color factor *R* is absent. How this slight pigmentation in the epidermal and hypodermal cells may occur is not clear, but in observed cases it was noted that the phenomenon was associated with a relatively thin mesocarp 35 μ to 50 μ in thickness. With a mesocarp of this thickness the nucellar color factor *B* apparently produces some pericarp color.

Data on seed-coat structure, the suggested genotype color factor constitution, and the actual seed color of the more important sorghum varieties are given in Table 1.

TABLE 1.—*Thickness of the various seed-coat structures, pigment location, superficial seed color, and possible color factorial constitution of representative sorghum varieties*

Sorghum variety	Thickness in microns of—				Location of pigment	Possible factors for color inheritance [a]	Notes
	Pericarp, including nucellar layer if present	Nucellar layer only	Pericarp				
			Mesocarp only	Epidermis, hypoderm, and cuticle			
Feterita	140	55	70	15	Nucellar	*Bsr*	Chalky-white pericarp.
Dwarf hegari	140	56	77	7	do	*Bsr*	Colorless pericarp with small reddish specks.
Manchu brown kaoliang.	140	35	70	35	Nucellar Pericarp	*BsR*	Walnut-brown pericarp.
Red Amber	110	60	10	40	do	*BSR*	Reddish brown pericarp.
Broomcorn	95	45	10	40	do	*BSR*	Amber to walnut-brown pericarp.
Kansas Orange	85	25	25	35	do	*BSR*	Walnut-brown pericarp.
Darso	80	30	28	22	do	*BSR*	Do.
Schrock	125	45	50	30	do	*BSR*	Do.
Sudan grass	30	15	Trace.	15	do	*BSR*	Chocolate-brown pericarp.
Pink kafir	78	3	50	25	do	*BSr*	Colorless pericarp with pinkish specks or blotches. See text regarding nucellar layer.
Dawn kafir (Dwarf Blackhull).	70	Absent.	40	30	Absent	*bSr*	Colorless pericarp with small reddish brown specks or blotches.
Sunrise kafir	80	Absent.	45	35	do	*bSr*	Do.
Reed kafir	75	Absent.	45	30	do	*bSr*	Do.
Red kafir	45	Absent.	20	25	Pericarp	*bSR*	English red pericarp.
Freed sorgo	65	Absent.	30	35	Absent	*bSr*	Colorless pericarp.
Shallu	50	Absent.	10	40	do	*bSr*	Do.
Yellow milo	65	Absent.	45	20	Pericarp	*bSR*	Ochraceous-salmon yellow pericarp.
White milo	75	Absent.	40	35	Absent	*bSr*	Colorless pericarp.

[a] *B*, a dominant factor for pigmented nucellar layer, and *b*, its allelomorph for absence of nucellar layer and color; *S*, a factor for vestigial mesocarp, and *s*, its allelomorph for a heavy, starchy, opaque mesocarp; *R*, a dominant factor for color in the epidermal and hypodermal cells of the pericarp, and *r*, its allelomorph for lack of color in this region.

GENETIC DATA

RED AMBER SORGO × FETERITA

The original Red Amber sorgo × feterita cross, with its reciprocal, was made in 1917, and the F_2 generation was grown by the writer in 1919, long before the seed-coat study reported above was made. In the F_2 generation the color intensities of segregates ranged from deep mahogany red or brown to white. Without a knowledge of the structure of the seed coat and the location of pigmentation it was difficult to classify the various colors properly. In this cross both parents possess a highly developed pigmented nucellar layer. The Red Amber sorgo differs from the feterita in that it possesses a pigmented pericarp with a poorly developed or vestigial type of mesocarp. The feterita has no pericarp color, but it has a highly developed starchy, opaque mesocarp that completely masks the pigmented nucellar layer.

The factors for seed color in line with Sieglinger's[11] findings may be designated in Red Amber as *BBSSRR*, and for feterita as *BBssrr*. The varieties, therefore, differ by the two factors *S* and *r*. The F_1 factorial constitution may be designated as *SsRr*. The 1,352 F_2 plants were classified as shown in Table 2.

TABLE 2.—*Segregation for seed color and suggested factorial constitution in the F_2 generation of the cross Red Amber sorgo × feterita grown at Hays, Kans., in 1919*

Item	Number of plants having—				Total number of plants
	Dark and light brown seeds	Buff seeds	Light buff, cream,[a] and pseudowhite seeds (with nucellar layer)	True white seeds	
Observed	771.0	260.0	321.0	None.	1,352
Calculated	760.5	253.5	338.0	None.	1,352
Deviation	10.5	6.5	17		
Factorial constitution	1 *SSRR* 2 *SsRR* 2 *SSRr* 4 *SsRr*	1 *ssRR* 2 *ssRr*	1 *SSrr* 2 *Ssrr* 1 *ssrr*		
Ratio[b]	9	3	4		

[a] The slight coloration in this group is believed to be due to nucellar pigmentation.
[b] $\chi^2=1.1667$; $P=0.5667$.

No true whites would be expected in this cross, since both parents possess the pigmented nucellar layer. When the original classification was made in 1919, seeds with a slight tint of buff or cream color were placed in the true-white group. This was, of course, incorrect, as will be shown in the analysis of the reciprocal cross feterita × Red Amber sorgo made at a later date. Only 1 individual (*ssrr*) out of 16 would be expected to have the pseudowhite seed color of the feterita parent. That this was correct was showh by segregation in the reciprocal cross feterita × Red Amber sorgo, as shown in Table 3.

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[11] SIEGLINGER, J. B. Op. cit.

TABLE 3.—*Segregation for seed color in the F_2 generation of the cross feterita × Red Amber sorgo, grown at Hays, Kans., in 1919*

Item	Number of plants having—			Total number of plants
	Colored seeds	Pseudowhite seeds	True white seeds	
Observed	1,016	74	None.	1,090
Calculated (15 : 1)	1,022	68	None.	1,090
Deviation	6±7.99	6±7.99		

Microscopic examination was made of the seed coats of kernels of the several phenotypes of the Red Amber × feterita cross and of the parents. The relation of the mesocarp thickness to color in the several phenotypes, all of which possess the nucellar layer, is shown as follows:

Phenotype	Thickness of mesocarp (μ)	Phenotype	Thickness of mesocarp (μ)
Red Amber sorgo parent	10	Buff	45
Feterita	70	Light brown	45
Pseudowhite	70	Dark brown	25
Light buff	40		

Classification for the reciprocal cross was more exact than for the original cross in 1919. On the basis of this more exact separation there were 15 plants with colored seeds to 1 plant with pseudowhite seeds. Since the microscopic examination of the seed coats of the different phenotypes possessing a nucellar layer showed that the pseudowhite color of feterita is due to a well-developed starchy mesocarp, the genetic data show that such a mesocarp is inherited as a recessive character.

The observed number of plants having pseudowhite seeds very closely approximated the theoretical number. As previously pointed out, no true whites could be expected in this cross. These data confirm those of Vinall and Cron[12] for the same crosses. These workers state that difficulty was experienced in properly classifying the colored heads.

The F_3 generation of the Red Amber sorgo × feterita progeny was grown and classified for color segregation. The F_2 seed had been inoculated with spores of kernel smut in order to study the inheritance of smut resistance, and as a result many of the heads were destroyed. This reduced the number available for color classification. Some of the F_2 heads that had been classified as white produced slightly colored seed in the F_3 generation. Likewise some F_2 heads that had been classified as browns and buffs produced a small proportion of whites in the F_3 generation, as would be expected from the segregation of heterozygous material. (Table 4.)

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[12] VINALL, H. N., and CRON, A. B. Op. cit.

TABLE 4.—*Expected F_3 breeding behavior of the cross Red Amber sorgo × feterita*

F_2 phenotype and frequency	Genotype frequency	Genotype	Expected F_3 breeding behavior
9 brown	1	*SSRR*	Brown (intensely colored), breeding true.
	2	*SsRR*	3 brown, 1 buff.
	2	*SSRr*	3 brown, 1 light buff.
	4	*SsRr*	9 brown, 3 buff, 3 light buff or cream, and 1 pseudowhite.
3 buff	1	*ssRR*	Buff, breeding true.
	2	*ssRr*	3 buff, 1 pseudowhite.
3 light buff and cream	1	*SSrr*	Light buff, breeding true.
	2	*Ssrr*	3 light buff or cream, 1 pseudowhite.
1 pseudowhite	1	*ssrr*	Pseudowhite, breeding true.

The color of kernels classified as light buff, salmon, pinkish, and cream is believed to be due to the presence of both the nucellar factor *B* and the *S* factor for the thin mesocarp.

FREED SORGO × FETERITA

The F_2 generation of a cross between Freed sorgo and feterita was grown at the Hays station in 1926. The seeds of this cross were classified in the field during the fall of 1926 before a study had been made of pigment location. Seed colors fell into three phenotypes, none of which was highly colored. The most intensely colored seeds observed were described as light buff, others as buff white and a third group as white. An interesting feature of the buff-white group was the fact that the lower two-thirds of the seed, which was protected by the glumes, was white. The outer or upper end of the seed, which was more exposed to sunlight, was buff in color.

TABLE 5.—*Segregation for seed color and suggested factorial constitution in the F_2 generation of the cross Freed sorgo × feterita grown at Hays, Kans., in 1926*

Item	Number of plants having—				
	Light buff seeds	Buff white seeds	Pseudo-white seeds (with nucellar layer)	True white seeds (without nucellar layer)	Total number of plants
Observed	235	168		301	704
Calculated	220	176	132	176	704
Deviation	15	8			
Factorial constitution	1 *BBss*	4 *BbSs*	2 *Bbss*	1 *bbss*	
	2 *BBSs*		1 *BBss*	2 *bbSs*	
	2 *BbSS*			1 *bbSS*	
Ratio [a]	5	4	3	4	

[a] The plants having pseudowhite and white seeds were grouped together and the values for χ^2 (=1.5454) and *P* (=0.4764) determined on the basis of a 5:4:7 ratio.

As shown in Table 5, a Freed sorgo seed is without a nucellar layer or pigmentation in any region, and it has a thin mesocarp. The only source of coloration in either parent of the cross is the highly colored nucellar layer of the feterita parent. In feterita this coloration is obscured by a well-developed mesocarp. The nucellar layer in the absence of a well-developed mesocarp and of pericarp color may cause seed colors ranging from light buff or pink to cream.

The genetic factors for seed color in the Freed sorgo are designated as *bbSSrr* and for feterita *BBssrr*. The recessive factor *r* is common to both parents. As shown in Table 5, the segregation in the F_2 generation for the two remaining factors was in the simple dihybrid ratio of nine plants with colored seeds to seven plants with white seeds. The observed segregation was in accord with the theoretical.

DISCUSSION

It frequently has been stated that normal characters are the result of the interaction of many factors plus environment. The effect of such interaction is strikingly demonstrated in the case of the three factors (*B*, *S*, and *R*) responsible for the inheritance of color in the seed of sorghum. When all three factors are present the pericarp is intensely colored. If the nucellar layer is present in combination with a highly developed mesocarp the nucellar color is invisible. To this extent a thick mesocarp functions as a color inhibitor. A very thin mesocarp makes it possible for nucellar pigmentation to be observed through the pericarp, and there is some indication of slight pigmentation in the pericarp itself even when the pericarp color factor is absent. The mesocarp was entirely absent in a few specimens examined.

The data indicate that mesocarp development is dependent on a single main pair of factors. A highly developed thick mesocarp is a recessive character. The extent of mesocarp development is somewhat variable, but whether this variation is the result of environmental influences or modifying genetic factors is not known. The extent to which environment might influence the expression of seed color in sorghums is apparent from even casual observation. For instance, in a year of extreme drought mesocarp development might easily be less than in a year of sufficient moisture. Such a reaction would have a direct influence on the intensity of seed color in so far as this is determined by mesocarp thickness.

Whether the pigment found in the nucellar layer is of the same chemical origin as that found in the epidermal and hypodermal cells in the pericarp was not determined. Genetically two different factors are responsible for the development of color in the two regions, one acting in the nucellar layer and the other in the pericarp. The data from crosses in which the pigment was located in the nucellar region seem to indicate that slight pigmentation also occurred in the pericarp of progenies in which the mesocarp was relatively thin. This would indicate at least some slight effect of the *B* factor on the epidermal and hypodermal cells of the pericarp when a thick mesocarp is not present to act as an inhibitor. That the two color factors are distinct is shown, however, when a sorghum such as Yellow milo with a colored pericarp is crossed with a variety lacking pigmentation in any region. In such cases seed color is inherited in a monohybrid ratio.

Reference has been made in Table 1 to certain varieties of kafir and Dwarf hegari that are white or colorless with small reddish brown specks or blotches. These specks are a definite varietal characteristic. Microscopic examination of Blackhull White kafir seed in areas where the specks occurred showed small but scattered remnants of pigmented nucellar cells. In sections taken a little to one side of the specks there were no nucellar cells. It was also noted that the starchy mesocarp cells were very poorly developed just above the nucellar remnants, so that the coloration of the remnant groups was highly apparent.

Dwarf hegari has a fully developed nucellar layer, but the seed coat is more or less specked. In this variety the mesocarp was more poorly developed in the areas where the specks occurred than in sections taken a little to one side of the coloration.

A peculiar condition was found in some strains of Pink kafir in that a faint remnant of the nucellar cell walls surrounded the entire endosperm. The thickness of this remnant wall averaged approximately 3 μ, while the thickness of the nucellar layer in most varieties measured from 25 μ to 50 μ. The seed coat of Pink kafir is colorless except for pinkish specks or blotches. These specks apparently are due to isolated groups of nucellar cells overlaid by a thin mesocarp similar to those occurring in the white-seeded kafirs.

The inheritance of specks on the seed coat has not been determined.

The writer was not able to determine any relation of the aleurone layer or the endosperm to color development in sorghum. The cells of the aleurone layer in all sorghums are relatively small and like the endosperm are without color. The aleurone and endosperm color factors so prominent in corn have no apparent analogy in sorghum.

A knowledge of seed structure and its relation to the inheritance of seed color should aid in classifying and in determining the origin of the numerous sorghum hybrids that are constantly arising under field conditions.

SUMMARY

The inheritance of seed color in sorghums has been perplexing. A knowledge of seed-coat structure and the location of pigments in sorghum kernels as determined by microscopic observations gives a better basis for interpretation than has been available heretofore.

Pigmentation may occur (1) in the epidermal and hypodermal cells of the pericarp, (2) always in the nucellar layer when this structure is present, or (3) in both regions at the same time. The nucellar layer does not occur in all varieties.

B is assumed to be a factor the presence of which is responsible for the development of the nucellar layer and its associated pigment. Its recessive allelomorph, *b*, determines the absence of a nucellar layer and a consequent lack of any color due to nucellar pigmentation. There is some evidence that the *B* factor may cause a slight coloration in the epidermis of the pericarp when in combination with the factor *S*.

S is assumed to be a factor determining the development of a vestigial type of mesocarp, its recessive allelomorph, *s*, determining a well-developed, starchy, opaque mesocarp. A thick starchy mesocarp masks nucellar color and inhibits even a slight expression of color in the pericarp due to *B*.

R is a factor determining coloration in the epidermal and hypodermal cells of the pericarp, *r* being its allelomorph responsible for the absence of color in the pericarp. *R* is greatly intensified in the presence of *B* and *S*. Slight coloration in the pericarp due to the nucellar factor, *B*, is independent in its inheritance from the pericarp color factor, *R*, the interaction of these two factors producing a more intensive color in the presence of *S*.

The thickness of the mesocarp is a determining factor in the external expression of nucellar coloration. When about 70 μ in thickness the mesocarp masks nucellar color and completely inhibits any slight color that might occur in the pericarp due to the factor *B*. The mesocarp ranges in thickness from nothing in certain unclassified sorghum hybrids to 70 μ or more in feterita. Environmental influences may alter the development of the mesocarp and affect the expression of pericarp pigmentation to a certain degree, but for the most part it is believed that the thickness of the mesocarp in any variety of sorghum is a fairly constant heritable character.

The nucellar layer is a remnant of the nucellus that was not absorbed during the development of the embryo and endosperm. In some varieties of sorghum the nucellar layer is entirely absorbed. It was never more than one cell thick in any variety studied, and in seeds of Pink kafir only a thin remnant of the outside wall of the nucellar layer was found. A group of scattered nucellar cells overlaid by a thin mesocarp is believed to be responsible for the specks found in the seed coat of certain white-seeded kafirs.

There apparently are no endosperm or aleurone colors in sorghums such as occur in corn.

THE RELATION OF SODIUM NITRATE AND CERTAIN OTHER NITROGEN CARRIERS TO THE DEVELOPMENT OF CHLOROSIS IN RICE[1]

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INTRODUCTION

The relative value of nitrate and ammonium salts as sources of nitrogen for rice has been the subject of numerous investigations. In general it has been the conclusion from such investigations that ammonium salts are of decidedly greater value than nitrate salts for this purpose.

Various explanations have been offered for the failure of nitrates, under comparable conditions, to produce growth and yields of rice similar to those produced by ammonia. Unfavorable soil reaction, unavailability of iron, accumulation of poisonous nitrites when nitrates are reduced in submerged soils, and denitrification have been offered as explanations of the unfavorable results secured with nitrate nitrogen. Various investigators have attempted also to determine the cause of the development of chlorosis in rice. It was for the purpose of securing further experimental evidence concerning these questions that the work here reported was undertaken.

EXPERIMENTAL METHODS

A study was made of soil reaction and of nitrogen changes in both greenhouse and field soils. Three series of cultures were studied in the greenhouse. The soil used was taken from the experiment station farm and is classified as Clarksville silt loam. It had not been cropped to rice previously.

Corn was grown on it the previous year. The soil was taken from a slightly eroded spot and was therefore very low in organic matter and total nitrogen. It is of limestone origin, but cropping and leaching have reduced its basic materials until it now shows an acid reaction.

The soil was taken from the field, screened, thoroughly mixed, and placed in glazed earthenware jars in the greenhouse. There it remained from January 20 to March 14. No water was added and no drainage was provided. Treatments were applied March 14 and the experiments started. Common vetch plants almost at bloom stage, cut into 1-inch segments and thoroughly mixed with the soil, were used for green manure. Chemically pure fertilizer

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[1] Received for publication Sept. 4, 1928; issued December, 1928. Contribution from the Department of Agronomy, Arkansas Agricultural Experiment station. Published with the approval of the director of the station. Research paper No. 104, Journal Series, University of Arkansas.

[2] The writers acknowledge their indebtedness to G. H. Banks, assistant director in charge of the Rice Branch Experiment Station at Stuttgart, Ark., for taking the field samples and for field observations.

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salts were applied by spraying on in solution, the soil being spread in a tub and shoveled over as the spray was applied.

The first series of jars was used for a study of nitrogen changes. The second series was devoted to a study of soil reaction and the third series was used to furnish a basis of comparison between various nitrate and ammonium salts in different concentrations.

In addition to the greenhouse work, field samples were taken at intervals from certain of the fertility plots at the rice branch station and analyzed. Nitrogen changes and the soil reaction were studied in these samples.

Water extracts were made of both wet and dry soils for nitrogen studies. When analyzing flooded soil, the excess water was poured from the sample and 60 gm. of the soil were weighed out for analysis. To this was added 250 c. c. of distilled water in a shaker bottle. With dry soil only 50 gm. were used. After 20 minutes agitation in a mechanical shaker the samples were filtered. Owing to the presence of considerable carbon dioxide in the samples of flooded soil and the consequent formation of carbonate in the extract calcium hydroxide did not give entirely satisfactory results as a flocculent. Sodium chloride was therefore substituted, as it was found that it did not interfere with or influence the ammonia, nitrite, nitrate, and total soluble nitrogen determinations, provided silver sulphate was used to precipitate the chloride in the nitrate determination as recommended by Harper (*4*).[3] After filtering aliquot portions were withdrawn for the various determinations.

Ammonia was determined by Nesslerization, nitrites by the Greiss colorimetric method, and nitrates by the phenoldisulphonic acid method. Total nitrogen was determined by the Kjeldahl method modified to include nitrogen as nitrates. Hydrogen-ion concentration was determined colorimetrically, using the drop-ratio method described by Gillespie (*3*). Water extracts in the ratio of 1:2 were made for the hydrogen-ion determinations by the dialysis method described by Pierre and Parker (*12*).

Rice of the Blue Rose variety was used throughout the experiment. The seed was germinated in silica sand with distilled water and transplanted to the jars when 3 to 4 inches high. Ten days after the plants were transplanted the soil was submerged.

NITROGEN CHANGES IN SOIL

The writers have shown in a previous publication (*6*) the nature of the nitrogen changes taking place in a submerged soil when cropped to rice and when uncropped. In the work reported here an attempt was made to determine how much nitrogen was available to the rice plants at various stages of growth and ascertain the form in which it existed. Twelve jars were used, 4 of which were treated with green manure (vetch) at the rate of 12 tons per acre, 4 with sodium nitrate at the rate of 250 pounds per acre, and 4 with ammonium sulphate at a rate sufficient to supply the same quantity of nitrogen applied to the sodium nitrate series. The green manure treatment was rather heavy and carried more nitrogen than the fertilizer salts, but the heavy application seemed desirable in order that a large amount of ammonia might be available in these jars. Two jars of each treatment were cropped and two were not.

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[3] Reference is made by number (italic) to "Literature cited," p. 601.

The rice plants were transplanted into the jars on March 16. All jars, cropped and uncropped, were flooded with distilled water 10 days later, March 26. Samples of soil were withdrawn from the jars at intervals during the experiment and analyzed for ammonia, nitrites, and nitrates. In certain of these analyses an exactly similar sample was extracted in the same manner and a 200 c. c. aliquot sample used for determination of total soluble nitrogen by the Kjeldahl method, modified to include nitrogen as nitrates. After distillation into standard acid the sample was Nesslerized in order that it might be strictly comparable to the ammonia determinations.

The first samples were taken from the dry soil just before the rice was transplanted, the second 25 days after the jars were flooded, the third 17 days later, and the fourth 15 days later. The results of these analyses are shown in Table 1. As in the previous work of the writers (*6*), nitrites were not present in significant quantities at any time, never exceeding 0.10 part per million parts of oven-dry soil. These results are, therefore, omitted from the table.

TABLE 1.—*Nitrogen as ammonia, nitrates, and total soluble nitrogen in variously treated soils sampled before and at intervals after flooding, together with the yield of rice straw in the cropped soil*

Jar No.	Treatment	First sampling, Mar. 16 (dry soil)		Second sampling, Apr. 20 (25 days after flooding)		Third sampling, May 7 (42 days after flooding)		Total soluble nitrogen	Fourth sampling, May 22 (57 days after flooding); parts per million of nitrogen present as—		Total soluble nitrogen	Yield or rice straw (dry weight
		Parts per million of nitrogen present as—										
		NH_3	NO_3	NH_3	NO_3	NH_3	NO_3		NH_3	NO_3		
												Grams
258-259	Green manure, cropped	4.4	13.9	6.7	(1)	6.2	(1)	8.8	5.2	(1)	10.4	153.3
260-261	Green manure, no crop	4.7	13.3	13.9	(1)	56.2	(1)	49.8	38.9	(1)	38.4	-------
262-263	$NaNO_3$, cropped	2.9	26.8	4.1	(1)	6.0	(1)	5.5	7.7	(1)	7.2	80.5
264-265	$NaNO_3$, no crop	2.9	27.2	8.1	(1)	10.2	(1)	14.4	8.6	(1)	12.1	-------
266-267	$(NH_4)_2SO_4$, cropped	10.6	11.8	4.2	(1)	6.1	(1)	15.2	4.7	(1)	8.9	108.0
268-269	$(NH_4)_2SO_4$, no crop	10.5	11.6	8.6	(1)	16.4	(1)	21.6	9.9	(1)	9.8	-------

[1] Trace.

The results here shown indicate that the reduction of nitrates is very rapid in flooded soils. Kelley (*8*) and Nagaoka (*9*) found that sufficient nitrites were produced from the reduction of nitrates to exert a toxic effect on rice. The results of the writers in this and previous work (*6*) substantiate those of Willis and Carrero (*15*) and indicate that nitrites are probably not significant at any time in the soils investigated.

When the amounts of ammonia are compared with the figures for total soluble nitrogen it appears that ammonia constitutes, in most cases, nearly all of the total soluble nitrogen. The methods used may have been partially responsible for the larger discrepancies, the reasons for which are not clear.

It seems probable that some of the differences, in certain cases at least, between the amount of ammonia and the amount of total soluble nitrogen may have been due to amino nitrogen. Panganiban (*11*) found quantities of amino nitrogen in flooded rice soils ranging from 8.82 to 10.72 p. p. m. Using 2 per cent sodium hydroxide solution for extraction as recommended by Potter and Synder (*13*) and as used by Panganiban, and with a Van Slyke apparatus for making the determinations, the writers found as much as 10.7 p. p. m. of amino nitrogen in the jars to which sodium nitrate had been added. How much of this nitrogen is soluble in water and available to the plants is a question that has not been investigated.

Chlorosis became manifest in the plants treated with sodium nitrate by the close of the fifth week. It became pronounced by the close of the sixth week and persisted for nearly five weeks, when the plants began to assume a normal color again. Chlorosis also appeared in the plants treated with ammonium sulphate but came later, persisted for a shorter time, and was never so pronounced. The plants treated with green manure never showed a chlorotic condition.

Rice straw yields from this series are shown in Table 1. Since soil which has not been cropped to rice previously very frequently produces a great deal of sterility, otherwise designated as "straight head," one jar of each set of duplicates was allowed to become dry for a period of a week just previous to heading. Straight head, however, was only partially controlled, and grain yields, therefore, were not secured. The straw yields are averages of duplicates, as straw yields were not appreciably affected by drying the soil. Each jar contained 10 plants.

TOTAL-NITROGEN STUDIES

So far as the writers are aware no experimental results have been presented to show definitely what becomes of the nitrogen in a nitrate salt when the nitrate is reduced in a rice soil, though Kelley (*8*) and Nagaoka (*9*) presented evidence indicating that some of the nitrogen may be lost. It has been assumed in many cases that it is lost as free nitrogen, while in others it is assumed to be only partially reduced, nitrites, ammonia, or lesser oxidized forms of nitrogen resulting.

In the experiment just reported an attempt was made to solve this problem by studying the changes in total nitrogen. It was soon discovered, however, that the nitrogen as nitrate constituted too small a portion of the total nitrogen to enable the writers to detect changes. Accordingly, on May 1, a 200 gm. sample of finely pulverized, oven-dry soil containing 2 p. p. m. of nitrogen as nitrate was placed in each of three shaker bottles of 475 c. c. capacity. To each of these samples was added sufficient pure nitrate of soda to supply 0.1003 gm. of nitrogen. After the addition of 120 c. c. of distilled water the bottles were shaken in a mechanical shaker for one hour and then a sample was quickly removed from each with a wide-tipped pipette for analysis. A similar sample was removed for determination of the amount of dry soil in the sample. Each bottle was vigorously shaken by hand before the removal of a sample.

After the samples were removed the level of the water was carefully marked on the bottles and they were placed in a laboratory where no chemicals were used in order that they might be kept from any ammonia in the air. A sample 1 c. c. in size was removed from each bottle at intervals in order to determine when all the nitrates were reduced, and each time the new level of the water was marked on the bottle. On July 12, samples were again analyzed for total nitrogen. Not all the nitrates had been reduced, but a qualitative test showed far less nitrates than the samples originally contained. It seemed best not to let them stand longer as algae had begun to appear. The water in the bottles was carefully made up to the mark and sampling was done as before. In order to check the accuracy of the total-nitrogen determinations the nitrates were determined by the phenoldisulphonic acid method and subtracted from that originally put in the soil. The results are recorded in Tables 2 and 3.

TABLE 2.—*Change in the total nitrogen content of soil, treated with sodium nitrate, after standing submerged*

Sample No.	Total nitrogen on May 1	Total nitrogen on July 12
	Per cent	*Per cent*
1	0.087	0.053
2	.080	.055
3	.084	.052
Average	.0836	.0533

Average loss = 0.0303 per cent or 303 p. p. m.

TABLE 3.—*Nitrate content of soil, treated with sodium nitrate, after standing submerged*

Sample No.	Parts per million of nitrogen as NO_3
1	132.8
2	246.6
3	161.6
Average	[a] 180.3

[a] This figure deducted from the N originally in the soil as NO_3, 501.5 p.p.m., gives an average loss of 321.3 p. p. m.

The results secured indicate the nitrogen is lost when nitrates are reduced, the loss in total nitrogen agreeing fairly well with the loss shown by the nitrate determinations. Hence, when a nitrate salt has been applied to a rice soil and has been reduced it is probable that the plant will suffer from a lack of available nitrogen.

INFLUENCE OF NITRATE NITROGEN ON DEVELOPMENT OF CHLOROSIS AND PLANT YIELD

The results of fertilizer trials in the United States and other countries have indicated, in general, that a nitrogen fertilizer whose nitrogen is present as nitrate is inferior for rice fertilization to one in which nitrogen is present as ammonia. Palisoc (*10*) reported good results with ammonium nitrate in solution cultures, but under such conditions nitrates are not reduced as in submerged soil. Trelease and

Paulino (*14*) reported better results with ammonium nitrate than with any other form of nitrate in soil, but obtained still further improvement with a nitrogen equivalent application of ammonium sulphate. Willis and Carrero (*15*) suggested that the nitrate radical was probably somewhat toxic or in some way contributed to a chlorotic condition of the plant. They suggested further a possibility that the sodium ion in itself is toxic to rice. This latter explanation was suggested to the writers by some work with solution cultures. In order, therefore, to determine whether or not these factors are operative a third series of experiments was planned.

It was assumed that, if the nitrate radical or the sodium ion contributed to the chlorotic condition of the plants, an increased concentration of either should increase chlorosis. It was further assumed that if the sodium ion was toxic to the rice plant then sodium chloride should produce a toxic effect. The series was accordingly arranged in five groups, as follows: Group 1 (jars 300–303) had an application of ammonium sulphate, on the basis of 50 pounds per acre, applied to each jar. Jar 300 had no further treatment. Jar 301 received sodium nitrate in addition to the ammonium sulphate at the rate of 50 pounds per acre. Jar 302 was similarly treated except that the sodium nitrate was applied at the rate of 150 pounds per acre. Jar 303 had sodium nitrate applied at the rate of 450 pounds per acre in addition to the ammonium sulphate.

Group 2 (jars 304–307) was treated like Group 1 except that calcium nitrate in equivalent quantities replaced the sodium nitrate. Group 3 (308–311) was also similar, but with ammonium nitrate in quantities sufficient to furnish an equivalent amount of nitrate nitrogen. Group 4 (312, 313) received ammonium sulphate at the rate of 50 pounds per acre, but jar 312 had sodium chloride added at the rate of 250 pounds per acre and jar 313 had calcium chloride added in sufficient quantity to give an equivalent amount of chlorine. The fifth group received only sodium nitrate, jar 314 having an application equivalent to 250 pounds per acre and jar 315 an application equivalent to 500 pounds per acre.

Chlorosis appeared in all the plants in the series except the two receiving the heavier applications of ammonium nitrate. The chlorosis appeared about a month after the plants were transplanted into the jars and persisted for a period of about four weeks. Even the plants in the jars receiving the heavier ammonium nitrate application showed some chlorosis near the close of this period, though it was never pronounced. After the four weeks' period all plants again attained a green color and grew vigoroulsy.

The plants receiving the heavier applications of sodium nitrate showed no more chlorosis than those receiving the lighter treatment. Those receiving sodium chloride had the same appearance as those receiving only ammonium sulphate. In so far as chlorosis is concerned the sodium ion did not appear to be responsible, as similar conditions obtained where calcium nitrate was used and also where the smaller amounts of ammonium sulphate were applied. Neither would it appear, therefore, that the nitrate radical produced the chlorotic condition.

Willis and Carrero (*15*) and Kelley (*8*) observed chlorosis in rice plants in early stages of growth, but it disappeared as the plants became older. Willis and Carrero reported chlorosis in proportion to the quantity of nitrate added. The writers' results do not substantiate these findings. In the ammonium nitrate group the heavier the applications the less chlorosis appeared.

The plants in this series were not allowed to go to maturity, but were harvested at the age of 106 days and the weights of dry straw recorded. The results are shown in Table 4. The terms "light" "medium," and "heavy" are used to designate the relative rate of nitrate application. Each jar contained six plants.

TABLE 4.—*Yields of rice straw on soil receiving different quantities of nitrate and ammonium salts*

Group No.	Jar No.	Treatment	Yield of rice straw (dry weight)
			Grams
1	300	$(NH_4)_2SO_4$	12.8
	301	$(NH_4)_2SO_4 + NaNO_3$ (light)	12.8
	302	$(NH_4)_2SO_4 + NaNO_3$ (medium)	13.5
	303	$(NH_4)_2SO_4 + NaNO_3$ (heavy)	17.8
2	304	$(NH_4)_2SO_4$	13.0
	305	$(NH_4)_2SO_4 + Ca(NO_3)_2$ (light)	11.8
	306	$(NH_4)_2SO_4 + Ca(NO_3)_2$ (medium)	12.3
	307	$(NH_4)_2SO_4 + Ca(NO_3)_2$ (heavy)	18.8
3	308	$(NH_4)_2SO_4$	14.4
	309	$(NH_4)_2SO_4 + NH_4NO_3$ (light)	15.0
	310	$(NH_4)_2SO_4 + NH_4NO_3$ (medium)	20.2
	311	$(NH_4)_2SO_4 + NH_4NO_3$ (heavy)	33.5
4	312	$(NH_4)_2SO_4 + NaCl$	13.3
	313	$(NH_3)_2SO_4 + CaCl_2$	13.5
5	314	$NaNO_3$ (medium)	10.8
	315	$NaNO_3$ (heavy)	15.0

EFFECT OF SUBMERGENCE ON HYDROGEN-ION CONCENTRATION OF SOIL AND PLANT YIELD

Willis and Carrero (*15*) at the Porto Rico station attempted to show the cause of the development of chlorosis in rice plants. Working with both calcareous and noncalcareous soil, they concluded that a lack of available iron was primarily responsible for the chlorosis. They thought it probable that the sodium ion in sodium nitrate, left as a residue in the soil when the nitrate was assimilated, was instrumental in inducing chlorosis. This, they said, was due to the change in reaction of the soil brought about by the basic residue.

The writers have observed chlorosis in rice plants in previous experiments. With these observations as a basis the second series of the present experiment was planned in such a way as to make it possible to study the relation of soil reaction and forms of nitrogen to chlorosis. It was designed to show whether soil reaction, involving the availability of iron, or the amount and availability of nitrogen was responsible for chlorosis. The soil, as stated previously, was not calcareous and its normal reaction was acid.

Very little work has ever been reported concerning the reaction of submerged soils. In those cases where such work has been reported (including previous work (*6*) by the writers) it was assumed that the reaction of the irrigation water was a reliable criterion of the reaction within the soil mass, and determinations of hydrogen-ion concentration were made, therefore, on samples of the flood water. In the present experiment samples of soil were removed from the jars at frequent intervals for determination of the hydrogen-ion concentration. The soil in all jars was kept flooded continuously until about 10 days before the last samples were taken, when one of the duplicate jars of each treatment was allowed to become dry in order to determine what change would take place in the reaction. Distilled water with a reaction of pH 6.5 was used for irrigating the plants. The numbers of the jars and the corresponding treatments are shown in Table 5.

TABLE 5.—*Hydrogen-ion concentration of variously treated rice soils before and at intervals after flooding*

Jar No.	Treatment	Hydrogen-ion concentration of soils on—									
		Mar. 21 (original)[a]	Apr. 2	Apr. 9	Apr. 16	Apr. 23	Apr. 30	May 14	June 5	July 10	July 25
		pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
270	NaNO$_3$ flooded, cropped	5.7	5.5	5.7	5.9	5.9	5.9	5.9	6.0	6.4	6.4
271	Same as 270, but drained at finish	5.75	5.7	5.7	5.75	5.8	6.1	5.9	5.9	6.5	[b] 5.6
272	NaNO$_3$+iron sulphate spray to leaves, flooded, cropped	5.7	5.5	5.8	5.85	5.9	5.9	5.75	5.8	6.4	[b] 5.65
273	Same as 272 but drained at finish	5.7	5.5	5.8	5.8	5.8	6.0	5.8	5.8	6.2	6.5
274	(NH$_4$)$_2$ SO$_4$, flooded, cropped	5.7	5.5	5.6	5.7	5.5	5.75	5.7	5.8	6.3	6.3
275	Same as 274, but drained at finish	5.7	5.6	5.6	5.6	5.5	5.7	5.7	5.7	6.1	[b] 5.7
276	No treatment, flooded, cropped	5.6	5.3	5.7	5.75	5.7	5.8	5.8	5.8	6.7	6.6
277	Same as 276, but drained at finish	5.75	5.5	5.9	6.1	5.8	5.8	5.7	5.9	6.5	[b] 6.0
278	No treatment, flooded, no crop	5.6	5.7	5.8	5.9	5.6	6.3	6.1	6.7	6.7	6.7
279	Same as 278, not drained	5.7	5.5	5.6	5.8	5.6	6.1	6.3	6.6	6.6	6.7
280	NaNO$_3$+sulphur, flooded, cropped	5.75	5.0	5.4	5.4	5.4	5.7	5.6	5.9	6.4	[b] 5.4
281	Same as 280 but drained at finish	5.4	5.0	5.1	5.4	5.2	5.6	5.5	6.1	6.4	6.3
282	NaNO$_3$ in 3 applications, sulphur, flooded, cropped	5.2	5.0	5.1	5.2	5.3	5.7	5.6	5.9	6.4	6.4
283	Same as 282 but drained at finish	5.5	5.0	5.1	5.3	5.3	5.6	5.75	6.2	6.4	[b] 5.2
284	NaNO$_3$+HCl to give reaction pH 4.0, flooded	4.0	5.1	5.1	5.2	5.2	5.7	5.5	6.3	6.0	6.1
285	Same as 284, not drained	4.0	5.1	5.2	5.4	5.5	5.6	5.7	6.2	5.9	6.1
286	NaNO$_3$ in 3 applications+HCl to give reaction pH 5.1, flooded cropped	5.1	5.4	5.5	5.4	5.5	5.6	5.6	5.7	6.5	6.3
287	Same as 286, but drained at finish	5.1	5.4	5.7	5.5	5.6	5.8	5.7	5.8	6.5	[b] 5.6
288	No treatment, no crop, not flooded	5.6	5.5	5.5	5.6	5.6	5.8	5.6	5.8	5.9	5.7
289	Same as 288	5.7	5.5	5.5	5.7	5.6	5.75	5.7	5.8	5.7	5.8
290	NaNO$_3$+HCl to give reaction of pH 5.1, flooded, cropped	5.1	5.2	5.2	5.3	5.2	5.4	5.4	5.8	5.9	6.3
291	Same as 290, not drained	5.1	5.6	5.3	5.3	5.2	5.5	5.2	5.5	5.7	5.7

[a] The figures in this column represent the pH values of the soils just prior to submergence.
[b] Allowed to become dry a week before determinations were made.

The sulphur in jars 280, 281, 282, and 283 was uninoculated and was applied to the soil at the rate of 900 pounds per acre two weeks before the plants were transplanted into the jars, and three weeks before the soil was submerged. Qualitative tests showed sulphates in these jars until 40 days after the plants were transplanted, at which time they seem to have been completely reduced.

Sodium nitrate was applied at the rate of 250 pounds per acre and, where not otherwise noted, in one application made before the plants were set into the jars. In jars 282, 283, 286, and 287 one-third was applied at this time, one-third when the plants were 47 days old, and one-third when they were 100 days old. Ammonium sulphate was applied at a rate sufficient to furnish nitrogen equivalent to that furnished by sodium nitrate.

In jars 284, 285, and 290, where hydrochloric acid was applied, the plants died. In jar 291 the plants lived to the close of the experiment, but made very poor growth. In jars 286 and 287 the plants grew well and matured, after being somewhat retarded by the acid treatment at the beginning of the experiment.

The results of the hydrogen-ion determinations are shown in Table 5. The first figure column represents the values for the dry soils just previous to submergence; the columns that follow show the values for flooded soil except where otherwise noted.

The data of Table 5 indicate that the flooding of the soil, in itself, changed the reaction decidedly toward alkalinity, the change being most rapid and attaining nearest the neutral point in jars 278 and 279 in which the soil was given no treatment, was flooded, and not cropped. The plants apparently tended to prevent this change as shown by a comparison of jars 276 and 277 with 278 and 279 and also jar 291, in which the plants lived, with jar 290, in which the plants died because of the application of hydrochloric acid. This tendency was probably due to the carbon dioxide excretions of the roots of the plants. Jacobson (*5*), starting with a culture solution having a reaction of pH 5.0 found that the rice plants changed the reaction to pH 3.0 during a period of three days. He believed root excretions of CO^2 were partially responsible for the change.

The change of reaction in the jars treated with ammonium sulphate was somewhat slower and did not attain quite the extent of that in the jars treated with sodium nitrate or in those that received no treatment. The difference was not marked, however. Even the sulphur and hydrocholoric acid treatments failed to prevent the change toward alkalinity.

When a number of the jars were allowed to become dry before the last samples were taken the reaction quickly reverted to near the original value. It would appear, therefore, that the change in reaction toward alkalinity was due to some incompletely oxidized compounds in the submerged soil.

The plants in all jars, except those treated with ammonium sulphate, began to show chlorosis as early as 35 days after the soil was submerged. At the end of 42 days the chlorosis was marked. At this time the reaction of the soil in the various jars ranged from pH 5.2 to pH 6.3, the least acid reaction of any cropped soil being pH 6.0, in one of the jars treated with sodium nitrate. After five weeks the plants began to show better color again and thereafter displayed no chlorotic symptoms. The plants in the jars treated with ammonium sulphate showed some chlorosis but it appeared later, lasted for a shorter period, and never became pronounced.

Sprayings of iron sulphate on the leaves of the plants in jars 272 and 273 failed to overcome chlorosis. An application of ferric citrate to the flood water in these jars likewise failed to improve the color of the plants. The second application of sodium nitrate, however, to jars 286, 287, 282, and 283 greatly improved the color of the plants and they never again showed marked chlorosis.

Rice yields from this series are shown in Table 6. The yields are the average of duplicates. Each jar in this series contained six plants.

TABLE 6.—*Rice straw yields from the soils used in studying soil hydrogen-ion concentrations*

Jar No.	Treatment	Yield of rice straw (dry weight)
		Grams
270–271	$NaNO_3$	62.5
272–273	$NaNO_3$+iron spray	55.9
274–275	$(NH_4)_2SO_4$	65.7
276–277	No treatment	51.8
280–281	$NaNO_3$+sulphur	61.8
282–283	$NaNO_3$ in 3 applications + sulphur	67.9
286–287	$NaNO_3$ in 3 applications + HCl	73.5
291	$NaNO_3$+HCl	15.5

FIELD STUDIES

In order to compare the changes in reaction and the nitrogen variations taking place under field conditions and in old rice soil with those observed in the greenhouse work, samples from certain of the fertility plots at the Rice Branch Experiment Station were analyzed. The soil of these plots is a typical rice soil of the Crowley series. The samples were taken in each case at such time as they could be shipped to the laboratory and analyzed the following day. While this arrangement was admittedly not so satisfactory as could be desired, owing to possible changes within the soil samples during transit, it apparently gave fairly reliable results. The samples were analyzed in the same manner as the greenhouse samples. The first samples were taken immediately after the ground was broken and before fertilizers were applied, the second after the fertilizers were applied and before irrigation began, the third four weeks after the soil was submerged, and the fourth eight weeks after submergence.

The rice station has been established rather recently and the plots employed in this work were used only one year previously for experimental work. The treatment given the various plots is shown in Table 7.

TABLE 7.—*Fertilizers added to experimental plots of rice soil at the Rice Branch Experiment Station*

Plot No.	Ammonium sulphate	Cottonseed meal	Superphosphate	Muriate of potash	Nitrate of soda	Hydrated lime
	Pounds per acre	*Pounds per acre*	*Pounds per acre*	*Pounds per acre*	*Pounds per acre*	*Pounds per acre*
15	66	190	166	53		
16	132		331	53		
17 [a]						
18		380	273	40		
19			331	53	177	
20	58 (urea)		331	53		
21 [a]						
24						1,325
25 [a]						
26	132		331	53		1,325
28			331	53	177	1,325
29	58 (urea)		331	53		1,325

[a] This soil was given no treatment.

The nitrogen and soil reaction data for the samples from these plots are given in Table 8. Nitrite nitrogen was never present in significant amounts, the highest concentration being slightly less than 0.1 p. p. m. in plot 28. These values are, therefore, omitted from the table.

TABLE 8.—*Nitrogen as ammonia and nitrates and pH of variously fertilized soil from the rice station fertility plots*

Plot No.	First sampling (before fertilization)			Second sampling (after fertilization)			Third sampling (4 weeks after flooding)			Fourth sampling (8 weeks after flooding)		
	Parts per million of nitrogen present as—		pH	Parts per million of nitrogen present as –		pH	Parts per million of nitrogen present as—		pH	Parts per million of nitrogen present as—		pH
	NO_3	NH_3		NO_3	NH_3		NO_3	NH_3		NO_3	NH_3	
15	5.6	7.2	6.4	46.0	2.8	6.4	10.5	8.1	7.0	(1)	12.1	6.75
16	6.0	5.0	6.4	31.2	(1)	5.9	11.4	10.3	7.0	(1)	8.3	6.7
17	14.6	6.6	6.2	10.2	(1)	6.4	10.8	12.4	6.8	(1)	29.8	7.0
18	5.1	6.1	6.4	9.8	2.3	6.2	36.1	9.5	7.3	(1)	12.4	7.0
19	10.0	6.9	6.3	28.4	1.8	6.2	16.2	11.0	7.2	(1)	7.8	6.8
20	12.9	4.4	6.7	11.4	2.0	6.0	11.7	10.5	6.9	(1)	27.6	6.8
21	10.0	6.7	6.3	9.4	2.1	6.0	11.7	12.3	6.9	(1)	8.1	6.8
24	13.5	9.3	6.1	17.6	2.6	7.7	10.1	7.8	7.3	(1)	11.6	7.1
25	5.5	8.5	6.1	9.8	(1)	6.0	13.7	6.1	6.9	(1)	8.3	6.9
26	9.2	4.8	6.5	23.4	(1)	7.6	18.9	11.3	7.2	(1)	9.2	6.9
28	14.0	7.4	6.5	32.2	2.0	7.7	17.3	9.7	7.3	(1)	23.5	6.9
29	25.0	6.2	6.1	19.0	1.8	7.3	10.5	8.4	7.2	(1)	27.7	7.0

[1] Trace.

None of the plants showed chlorotic symptoms except those in the four limed plots. These showed some chlorosis, which persisted for a considerable period. In plots 18 and 19, where the reaction became slightly alkaline, the plants retained a healthly, green color.

The soil in these plots contained a rather large amount of organic matter, much more than did the soil used in the greenhouse work. This high organic content made possible a comparatively high rate of ammonification as shown by the amounts of ammonia present in the soil within one month after submergence. These values were appreciably higher than for the cropped jars in the greenhouse at the corresponding sampling.

The irrigation water used on the field plots had a reaction of pH 7.3. Qualitative tests of the water showed that it contained small amounts of chlorides and sulphates, about 3.5 p. p. m. of nitrates, a trace of nitrites and ammonia, a large amount of bicarbonates, and a rather high content of salts precipitated by ammonia.

DISCUSSION

The evidence presented in the foregoing experiments indicates that insufficient available nitrogen, particularly ammonia, was responsible for chlorosis in the plants. That the chlorotic condition was not due to changes in soil reaction rendering the iron of the soil unavailable, as suggested by Willis and Carrero (*15*), was indicated by the following facts: (1) The reaction was pH 6.0, and lower in certain jars, when chlorosis became marked and iron was very probably not rendered unavailable with this reaction; (2) iron sulphate spray applied to the leaves of the plants failed to overcome chlorosis, as was true in the work of Willis and Carrero, though they thought its failure was due to failure of the spray to cover the leaves; (3) iron citrate added to the flood water had no remedial effect on the plants; (4) addition of the second application of sodium nitrate to certain jars during the period when the plants were most chlorotic greatly improved the appearance of the plants and they never again showed marked chlorosis; (5) where ammonium nitrate was added in heavy applications very little chlorosis appeared; (6) in those jars to which light applications of ammonium sulphate were made, chlorosis became pronounced; and finally (7) no chlorosis appeared where an application of 12 tons per acre of green manure had been made, due, very probably, to the steady evolution of rather large amounts of ammonia.

Gile and Carrero (*2*) observed chlorosis in rice on dry soil and found that it disappeared after the soil had been submerged. This disappearance they attributed to the development of roots under submerged conditions which were more capable of assimilating iron. Johnson (*7*), writing of this work, attributed the disappearance of chlorosis to the reduction of iron under submerged conditions from the ferric to the ferrous form, rendering it available to the plants. Willis and Carrero (*15*) further found that organic iron compounds were ineffective in preventing chlorosis. When bulky organic compounds such as stable manure, velvet-bean plants, and tobacco stems were used in considerable quantities, however, the condition was overcome, due, they thought, to the fact that the presence of these compounds enabled the plants to obtain more iron.

Much of the work of these investigators was done on calcareous soil and therein differed from the conditions under which the present writers worked. The results of the writers' experiments, however, would indicate that the reason the chlorosis observed by Gile and Carrero was overcome by submergence, was probably the evolution of ammonia after the soil was submerged. Ammonification undoubtedly took place in the dry soil, but the ammonia so produced was rapidly converted into nitrites and subsequently into nitrates. The writers have shown in a previous publication (*6*) that rice plants grow well in a dry soil if considerable ammonia is present, expecially in the early stages of growth. The bulky organic material added by Willis and Carrero probably greatly stimulated ammonification and thereby proved valuable also in overcoming the chlorotic condition. Arrhenius (*1*) has reported a "rather good rice soil" with a reaction of pH 7.0, with the significant statement that it was "very rich in humus." He expressed the belief that root excretions of carbon dioxide and other substances accumulate in the soil layer around the roots of rice plants where they act as amphoteric electrolytes changing the reaction in such manner as to enable the plant to maintain conditions favorable for growth.

The nitrogen of sodium nitrate or other nitrate salts is probably lost in the process of reduction, as indicated in this study, and the plant becomes chlorotic due to a lack of nitrogen before ammonification has progressed sufficiently to enable it to secure enough nitrogen. As ammonification proceeds the condition is overcome. Where large amounts of organic matter are present, as in the soil treated with green manure and the field soil used in this work, severe chlorotic conditions are probably not to be expected.

In the light of these investigations, sodium nitrate, or other nitrate salts can hardly be recommended for rice fertilization. If used, it would appear most economical to apply it in several applications in order to minimize the loss from denitrification.

SUMMARY

A study of the effect of sodium nitrate upon the reaction of rice soils, and upon nitrogen changes in such soils, has been reported in this paper. Soils treated with green manure and ammonium sulphate were also studied for purposes of comparison. Both greenhouse and field soils were used in the work.

Sodium nitrate and other nitrates were rapidly reduced in submerged soil. The experimental results indicate that nitrogen was lost in the reduction process by denitrification. Ammonification progressed slowly for the first four or five weeks following submergence except where an abundance of organic matter was present. Consequently, the plants suffered from lack of available nitrogen and became chlorotic. Chlorosis was overcome as ammonification progressed.

The soil reaction was changed toward alkalinity by flooding. Sodium nitrate had a slight tendency to hasten this change and ammonium sulphate tended slightly to retard it. Chlorosis became marked in nearly all cases before the soil reaction reached pH 6.0. An impaired availability of iron due to an alkaline reaction of the soil very probably was not, therefore, the cause of chlorosis.

Where an abundance of organic matter was present in greenhouse soil and in field soil chlorosis did not appear. This was due, very probably, to ammonia liberated from the organic matter. The reaction of the field soil reached pH 7.0 and slightly above in some cases. Where lime was applied to field soil some chlorosis appeared.

Spraying ferrous sulphate on the leaves of chlorotic plants failed to correct the condition. The addition of ferric citrate to the flood water for this purpose likewise failed.

A second application of nitrogen, while the plants were chlorotic, greatly improved their color and vigor. This appears to be good evidence that chlorosis was due to lack of available nitrogen, particularly lack of ammonia in early stages of growth.

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STUDIES OF THE PHOTOPERIODISM OF SOME ECONOMIC PLANTS[1]

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INTRODUCTION

In the spring of 1924 a study of the effect of certain seasonal day lengths on some economic plants was undertaken at the Porto Rico Agricultural Experiment Station at Mayaguez. At this latitude, 18° 12′ N., the time between sunrise and sunset ranges from 11 hours in December to 13.2 hours in June. In neither December nor June is the variation in day length within the month greater than six minutes.

METHODS OF EXPERIMENTATION

For the purpose of the test the plants were grown in three groups, under respectively a long, a short, and the normal daily light exposure. For the long exposure, electric lights were automatically switched on at sunset and off at 13½ hours after sunrise, thus furnishing a lighted period approximating in length that of June days at this latitude. Five 50-watt mazda blue "daylight" bulbs were set 2½ feet apart in a row above the containers in which the plants were grown, the distance from the bulbs to the soil surface being not more, and usually considerably less, than 5 feet. At the soil surface the intensity of illumination was 5½ to 6 candlepower, and half way to the lamps it was 15 candlepower.

The plants receiving the short daily light exposure were placed on cars which were run into a dark room in the latter part of the night and brought out into the light at 11 hours before sunset. This light exposure in length approximately corresponded to December days at this latitude, differing, however, in having but the single daily twilight.

Sweet potatoes, onions, pineapples, and beans were grown under the daily light exposures outlined above. In addition, the onions, pineapples, and potatoes were grown under daily light exposures, differing from the normal in slightly more exaggerated degree, of 10 and 15 hours' duration, respectively, and corn was grown under a 15-hour exposure. Though the duration of exposure to light is referred to in terms of hours with the sun above the horizon or the lighted period artificially protracted, each period also included in addition the single brief tropical twilight exposure.

For corn and for some of the plants which were grown in containers as well, a garden plot was electrically illuminated from sunset to 15 hours after sunrise. Five rows of lights were placed 5 feet apart. Each row contained four 40-watt lamps spaced 6½ feet apart. The lights were set 3 feet above the soil surface at planting and so remained over the low-growing plants, but were gradually raised over the

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tall-growing plants, such as corn, as growth made it necessary. At the surface of the ground the intensity of illumination ranged from 3.4 or 3.5 to 5 candlepower over the beds of low-growing plants. The intensity of illumination at the base of the tall corn was reduced to 1 or less candlepower after the ultimate lifting of the lights.

The check plants were grown in a plot that was sufficiently removed from the treated plot to be unaffected by the light from it.

PLANTS STUDIED

SWEET POTATOES

Two varieties of sweet potatoes, Porto Rico and Key West, were grown. Fourteen plantings were made between March 11, 1924, and June 30, 1925, at intervals of 4 to 8 weeks. The containers used were 5-gallon tin cans, each carrying river loam in amount equivalent to 50 pounds of moisture-free soil. At each planting the draws or slips for the different groups were derived from a single tuber. The vines were trained on wire screening so placed as to utilize the illumination to the best advantage. The first 2 plantings were run for 16 and 20 weeks, respectively, and subsequent plantings for 24 weeks. In addition to the plants which passed the entire period without change of light exposure, in 3 plantings other plants, after having passed half the period under either the long or the short daily light exposure, were transferred to the other exposure. Blossoming and production of vine growth and tubers were noted.

The shorter daily light exposure proved to be more favorable to the blossoming of both varieties. Four plants of the Porto Rico variety blossomed, and a fifth budded under the shorter exposure, two blossomed under the normal, and none whatever under the longer exposure. Of the Key West variety, 8 blossomed under the shorter, 6 under the normal, and 3 under the longer daily light exposure.

The evidence as to the effect of length of daily light exposure on vine growth and tuber formation was inconclusive. The curves of both growth and production under the different light exposures follow the same general trend.

Special conditions modified the growth of one planting of Porto Rico and five plantings of Key West, thus necessitating their elimination from comparisons of vine growth and tuber production. There remained for purposes of comparison 22 individuals under each light exposure. The behavior of the two varieties did not differ essentially and their data are combined in Table 1.

TABLE 1.—*Vine growth and tuber production of sweet potatoes under 11-hour, normal, and 13½-hour daily light exposures*

Length of daily light exposure	Data from 13 individuals under each light exposure			Data from 22 individuals under each light exposure					
	Length of vine growth alive on removal	Length of vine growth dead on removal	Total length of vine growth on removal	Weight of vine growth	Weight of tubers	Weight of tubers plus other roots recovered	Number of tubers weighing 100 or more grams	Number of tubers weighing 50 to 99 grams	Number of tubers weighing 1 to 49 grams
	Inches	*Inches*	*Inches*	*Grams*	*Grams*	*Grams*			
11 hours	1,885	918	2,803	2,131	4,839	5,257	18	16	57
Normal	1,887	743	2,630	3,450	4,855	5,516	18	13	41
13½ hours	1,833	639	2,472	2,943	4,975	5,454	17	17	40

The uniformity under the different light exposures was notable in respect to length of vine growth, total weight of tubers, and grading of tubers as to size. Too much importance should not be attached to the fact that the weight of vine growth was considerably less under the short light exposure, since the weight of vine growth was greater under the normal exposure than under the long exposure in 13 of 22 comparisons.

ONIONS

Four varieties of onions, Prizetaker, Bermuda White, Yellow Globe Danvers, and Silver King (Giant White Tripoli), were grown under the different light exposures. Plantings were made at four-week intervals from August 22 to December 12, 1925, and again on January 7, 1927. Because of poor seed, Yellow Globe Danvers and Silver King (Giant White Tripoli) were eliminated from the first planting, and the latter from the second planting as well. The soil and containers were like those used for sweet potatoes. Suitable fertilizer was uniformly mixed with the soil prior to planting. The seedlings of the first planting were germinated under a normal light exposure and transplanted to the containers in the different groups at two weeks after seeding. In subsequent plantings the seed was planted in the permanent container and the resultant seedlings were kept under the different light exposures from the start. Five were left to develop in each container.

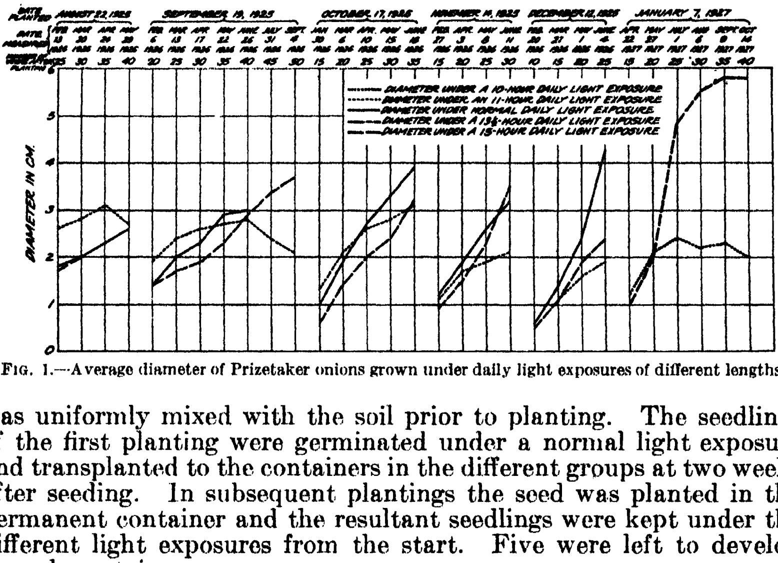

FIG. 1.—Average diameter of Prizetaker onions grown under daily light exposures of different lengths

Four or more measurements of the diameter of the bulb or pseudostem of each plant were taken at intervals of five weeks. The increase in diameter of the different varieties under the different light exposures is shown graphically in Figures 1, 2, 3, and 4.

The onions of two plantings were allowed to continue growth for considerably more than a year, whereas those of the other plantings were removed, weighed, and measured at 25, 30, 35, and 40 weeks after planting.[2]

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[2] Two varieties were removed on three occasions on the day prior to the termination of the week in order to facilitate note taking. This difference is disregarded.

PRIZETAKER

The Prizetaker under an 11-hour daily light exposure formed no bulbs,[3] the plants remaining in the spring-onion stage, with comparatively slender pseudostems, and green leaves. In the planting allowed to continue growth for the longest period, about 15 months, the stalk or pseudostem of these ancient spring onions measured 17 to 20 cm. from root base to the point of leaf spread, and only 1.2 to 1.4 cm. in diameter.

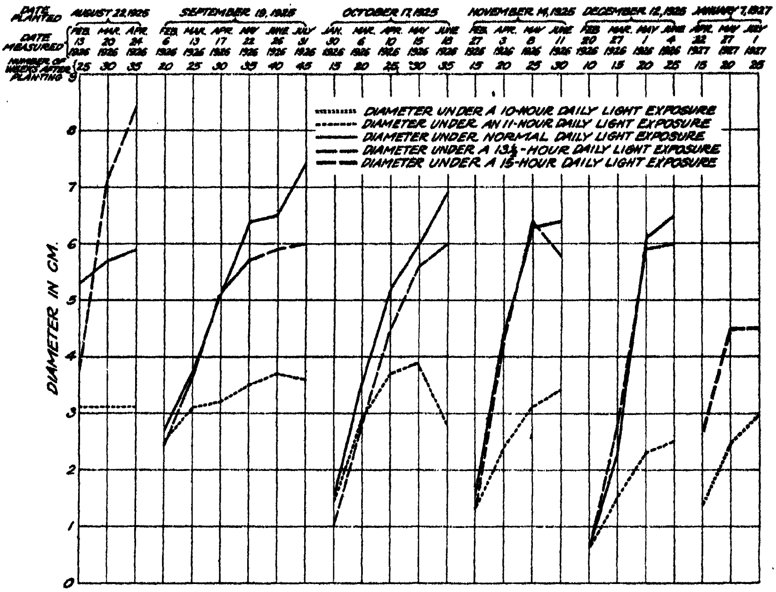

FIG. 2.—Average diameter of Bermuda White onions grown under daily light exposures of different lengths

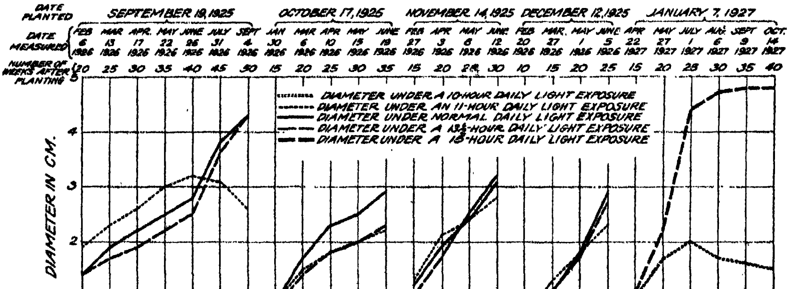

FIG. 3.—Average diameter of Yellow Globe Danvers onions grown under daily light exposures of different lengths

Under the normal light exposure there was some basal swelling of the pseudostems, but most individuals were still in the spring-onion stage. In the lot planted November 14 and removed June 11, one of the five plants had developed a well-formed bulb 5.8 cm. in diameter, and the tops, though still green, had fallen over on removal at 30 weeks, whereas the others in the container were in the spring-onion stage with erect turgid tops. In the lot planted December 12 and removed June 4, bulbs of considerable size had formed. Since with one exception the tops were still growing and were larger and more turgid than under the shorter light exposure, the stage of development appeared to be intermediate between that of spring onions and matured bulbs.

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[3] The term "bulb" in this discussion is not used in its botanical sense, but to designate the normal globular form of the resting onion, or a development approaching this.

Under the 13½-hour daily light exposure, as in the other groups, the development was mainly that of spring onions, although some were slightly swollen near the base. Of those planted December 12, one had developed a bulb comparable to those in the normal-day group, though smaller. At the time of removal there was little difference in duration of daily light exposure between the normal and the 13½-hour exposure. The relative proportions of tops to pseudostems or bulbs are given in Table 2 and show that the plants went chiefly to tops under daily light exposures of 11 to 13½ hours. (Fig. 5, A.)

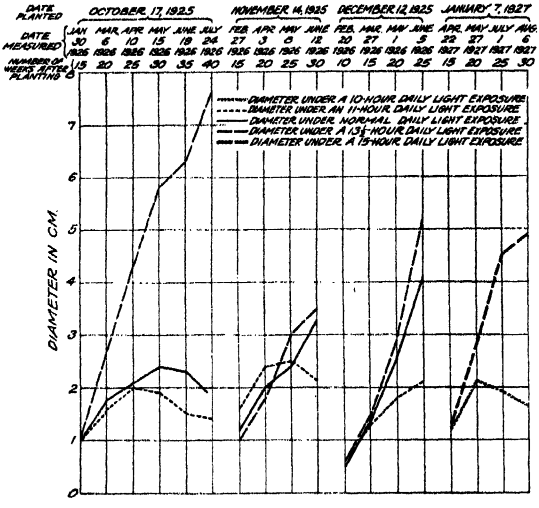

FIG. 4.—Average diameter of Silver King (Giant White Tripoli) onions grown under daily light exposures of different lengths

The plantings of January, 1927, were given different exposures from the preceding. Under a 10-hour daily light exposure all were still in a vigorously growing spring-onion condition at 60 weeks. In two instances, however, the basal portion of the pseudostem was slightly swollen. On the other hand, under a 15-hour daily light exposure, four bulbs had formed at 25 weeks and the tops of two had fallen over, an indication that they were approaching the rest period. Their condition in the twenty-ninth week is shown in Figure 5, B. At 30 weeks all had formed bulbs, and at 35 weeks the tops of three were dead and those of the others were dying. At 40 weeks the first of these had gone through the resting period and resumed growth.

There was no evidence as to any effect of length of daily light exposure on division or splitting of the individual into more than one. No individual of the planting of October 17 showed any splitting, whereas individuals in the following planting showed some splitting under the 11-hour, the normal, and the 13½-hour daily light exposures. On the whole, the splitting averaged the same for the three groups, one splitting into 1.2. In the single planting under a 10-hour exposure no splitting occurred; under the 15-hour exposure one of the five individuals had split into two prior to entering the rest period.

TABLE 2.—*Relation of tops[a] to bulbs or pseudostems of four onion varieties which were grown under 11-hour, normal, and 13½-hour daily light exposures*

Variety and portions weighed	Length of daily exposure	Average weight per plant of onions—					Total weight expressed in percentage of weight of tops
		Planted Aug 22, 1925, and removed 40 weeks later	Planted Oct. 17, 1925, and removed 35 weeks later[b]	Planted Nov. 14, 1925, and removed 30 weeks later	Planted Dec. 12, 1925, and removed 25 weeks later	Total weight	
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Prizetaker:							
Tops	11 hours	78	61	54	29	222	100
Pseudostems		51	42	33	19	145	65
Tops	Normal	68	66	60	53	247	100
Pseudostems or bulbs		31	60	59	55	205	83
Tops	13½ hours	75	66	75	57	273	100
Pseudostems or bulbs		36	43	46	27	152	56
Bermuda White:							
Tops	11 hours	59	30	17	31	137	100
Pseudostems or bulbs		48	57	60	37	202	147
Tops	Normal	20	4	1	1	26	100
Bulbs		185	125	103	97	510	1,961
Tops	13½ hours	1	7	0	0	8	100
Bulbs		229	89	84	81	483	6,038
Yellow Globe Danvers:							
Tops	11 hours	--------	57	67	53	177	100
Pseudostems		--------	29	35	28	92	52
Tops	Normal	--------	57	74	69	200	100
Pseudostems		--------	33	49	33	115	58
Tops	13½ hours	--------	66	81	61	208	100
Pseudostems		--------	29	43	26	98	47
Silver King (Giant White Tripoli):							
Tops	11 hours	--------	49	85	61	195	100
Pseudostems		--------	24	50	27	101	52
Tops	Normal	--------	--------	78	77	155	100
Pseudostems or bulbs		--------	--------	62	47	109	70
Tops	13½ hours	--------	28	57	65	150	100
Pseudostems or bulbs		--------	63	64	87	214	143

[a] Where a bulb had failed to develop, the division between top and pseudostem was made by cutting just below the spread of leaves.

[b] This applies to all except Silver King (Giant White Tripoli), which in this planting was left for nearly 61 weeks.

BERMUDA WHITE

Under the 10-hour daily light exposure the plants of the Bermuda White variety remained in the spring-onion condition. At 60 weeks there were 15 plants instead of the 5 original plants, but development had been made without prior bulb formation or a rest period. A slightly swollen base was the nearest approach to bulb formation.

Similarly, under an 11-hour daily light exposure the development for the most part was that of spring onions. Of those in the five plantings only one individual developed bulbs. On removal at 30 weeks after planting this had developed two poor bulbs, one of which contained two individuals.

In contrast to those under a shorter than normal light exposure, all plants grown under the normal light exposure developed normal bulbs. At 25 to 30 weeks these generally were entering or had entered the rest period. The first resumption of growth in two containers was at 35 and 39 weeks, respectively.

Under a 13½-hour daily light exposure, well-formed bulbs developed. On some of these the tops had fallen over at 20 weeks, and were dead at 22 weeks. In one instance growth was resumed at 27 weeks.

The plants under the 13½-hour and under the normal exposure, which in June closely approached the former in duration, showed in general a pronounced similarity in rate of increase in diameter.

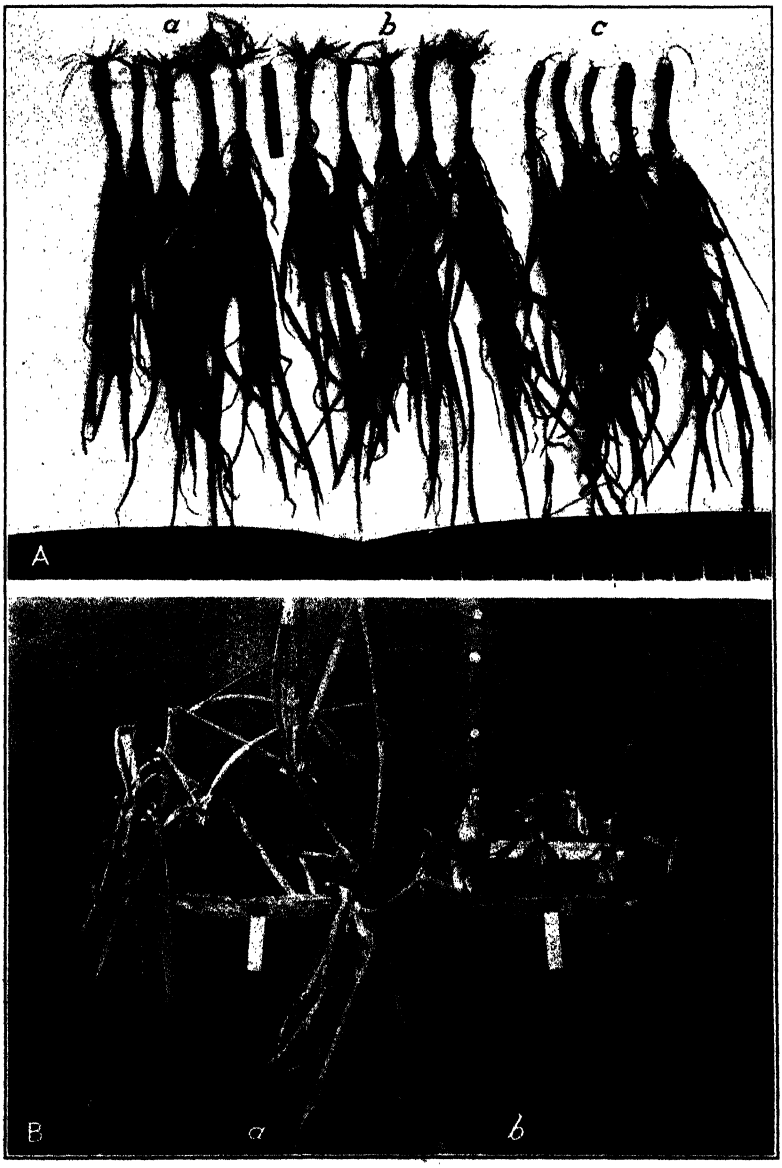

FIG. 5.—Prizetaker onions. A, Light exposures of *a*, 11 hours; *b*, normal; and *c*, 13½ hours. October 17 to June 18. B, Light exposures of *a*, 10, and *b*, 15 hours. January 7 to July 27

(See fig. 2.) The contrasts in proportional development under the different light exposures were striking. (See Table 2.) Figure 6, A, shows the development made in 25 weeks under the different exposures.

Under the 15-hour daily light exposure bulbs developed rapidly, though they were somewhat smaller. At 15 weeks four bulbs had developed and the fifth was intermediate in form. At 18 weeks the tops of these small, well-formed bulbs had fallen over but were still green. At 20 weeks the tops were dead or dying. At this time the leaf development of this lot was in striking contrast to that of the plants grown under a 10-hour exposure. The longest leaf per plant

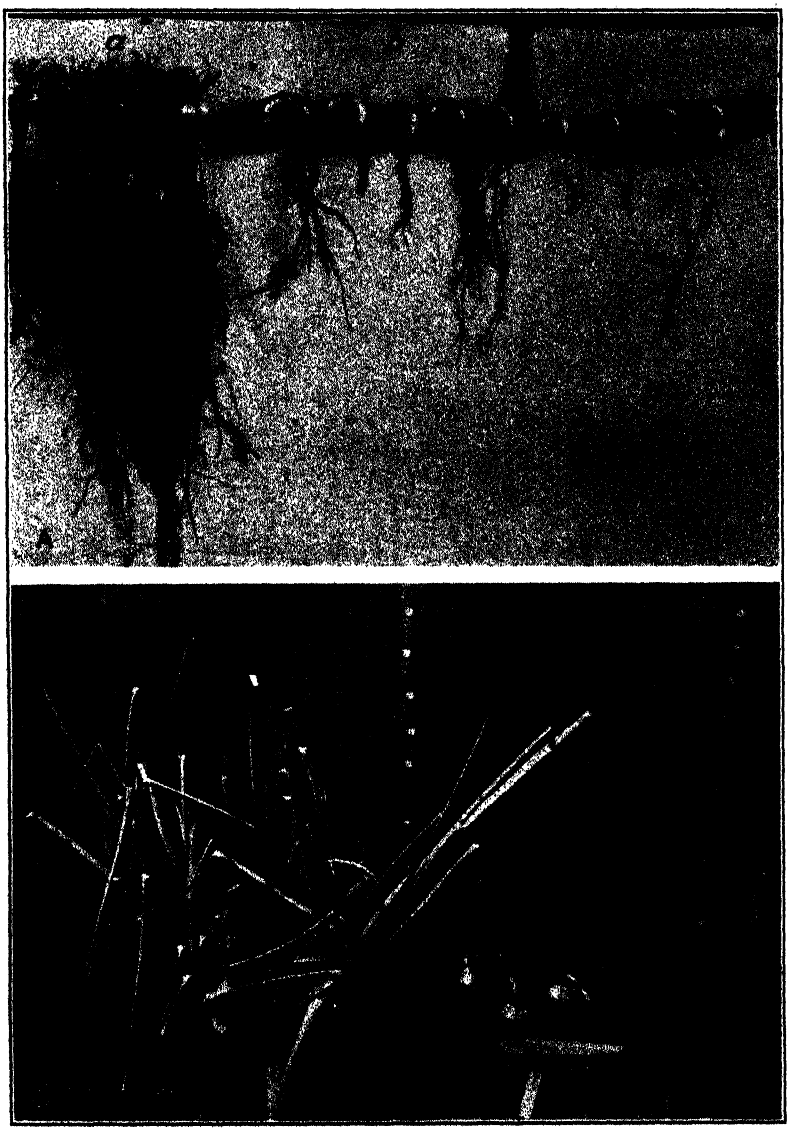

FIG. 6.—Bermuda White onions. A, Light exposures of *a*, 11 hours; *b*, normal; and *c*, 13½ hours. December 12 to June 4. B, Light exposures of *a*, 10, and *b*, 15 hours. January 7 to June 13

of the former averaged 20 inches in length, in contrast to an average length of 31.6 inches under the 10-hour exposure, measuring from soil surface to leaf apex. The condition of the plants in the twenty-third week is shown in Figure 6, B. At 25 weeks one plant had terminated the resting stage and resumed growth. After the remaining plants had resumed growth, all were removed but one, as they were infected with *Sclerotium rolfsii.* The remaining one resumed growth at 29 weeks. At 40 weeks five well-formed small bulbs had developed from it, and the tops had fallen over. At 42 weeks the plants had entered the resting stage and the tops were dead. At 45 weeks one plant had resumed growth, and at 50 weeks all were again growing. Thus, within the year two growth periods and two rest periods were terminated and a third period of growth was entered upon. At 60 weeks the plants had the appearance of vigorous young spring onions. No indication of blossoming appeared at any time.

Among the plants which had formed bulbs the development of more than 1 core was often shown on resumption of growth, 2 individuals arising from what had externally appeared to be a perfectly formed single-cored bulb. Of the plantings grown in 20 containers under the different daily light exposures, only 1 failed to show splitting of 1 or more individuals. Under the 11-hour exposure, splitting was in the proportion of 1 to 2.1, and under the 13½-hour exposure, of 1 to 2.2. At 35 weeks 13 individuals, 1 with 3 cores, had developed from the 5 plants under a 10-hour exposure, and 12 individuals, 1 with 2 cores, had developed from the 4 plants in the contemporary lot under a 15-hour exposure, the rest period of the latter having been terminated and growth resumed. Owing to vigorous top growth and the absence of bulb formation, the splitting of 1 individual into several was more conspicuous under the shorter exposures than under the longer daily light exposures, where bulbs were quickly formed and the rest period was entered. On resumption of growth, however, the compound formation under the longer exposures became evident.

YELLOW GLOBE DANVERS

Plants of the Yellow Globe Danvers variety under a 10-hour daily light exposure were still in the spring-onion stage at 60 weeks after planting.

Plants removed at 25 to 35 weeks after planting showed comparatively little difference in development under daily light exposures of 11 hours, normal length, and 13½ hours. (See Table 2.) All had green tops and long pseudostems. (Fig. 7, A.) The basal section of the pseudostem generally was more swollen under the normal exposure than under the 11-hour exposure. In the planting of December 12, 1 individual under the 13½-hour exposure progressed beyond the spring-onion stage and developed a swollen base. Otherwise, all were classed as spring onions.

The planting of September 19 was left for a longer period. At 45 weeks after planting, 1 or 2 plants in each container had rotted, and the others were still in the spring-onion stage, though under the 13½-hour exposure 1 of the 4 individuals showed a much swollen base. At 50 weeks the basal diameters averaged 2.6 cm. under the 11-hour exposure, and 4.3 cm. under the normal and the 13½-hour

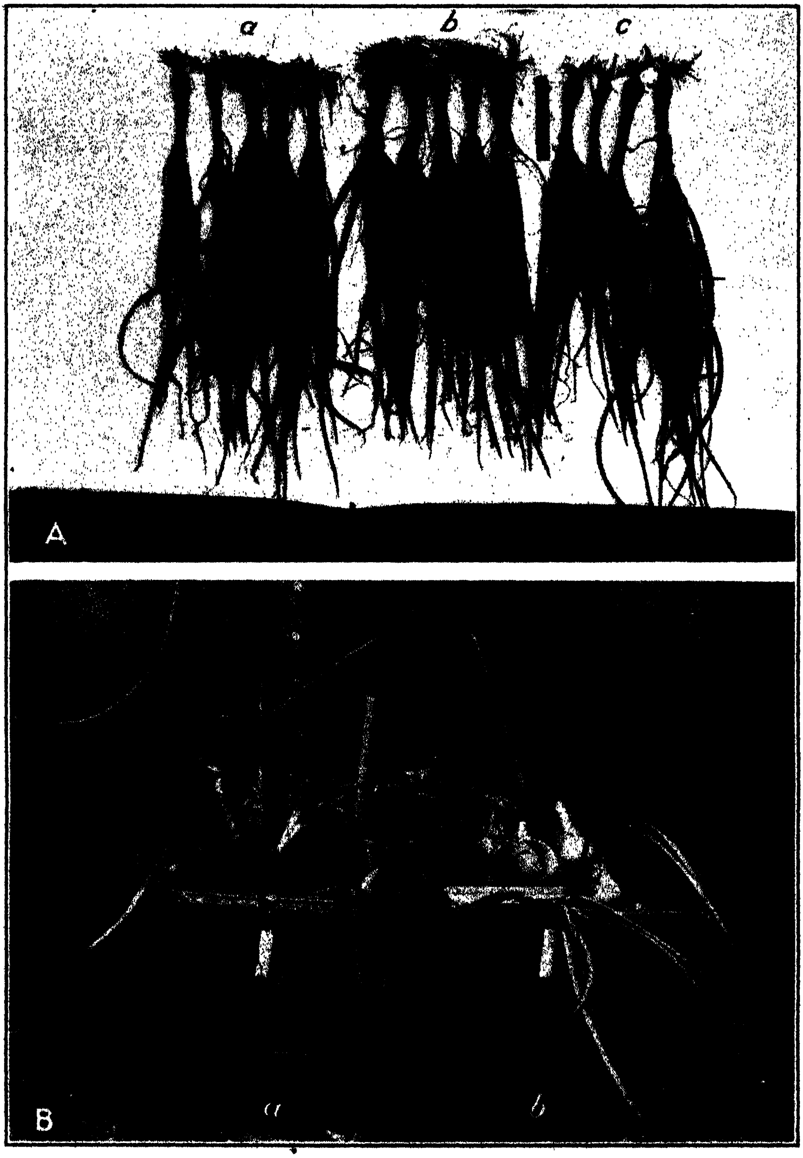

FIG. 7.—Yellow Globe Danvers onions. A, Light exposures of *a*, 11 hours; *b*, normal; and *c*, 13½ hours. October 17 to June 19. B, Light exposures of *a*, 10, and *b*, 15 hours. January 7 to July 26

exposures, showing the tendency of the 2 latter toward bulb formation. Rotting eliminated the normal-day group. The 3 individuals remaining under the 13½-hour exposure had formed small bulbs with tops over at 50 weeks, and tops dead at 51 weeks. Of these a single individual remained on removal at a little less than 65 weeks. It had gone through the resting stage and just resumed growth. The bulb weighed 23 gm. and measured 3.7 cm. in horizontal by 4 cm. in vertical diameter.

Under a 15-hour exposure bulb formation was rapid. At 25 weeks all had formed or were forming bulbs and the top of 1 was already over. The pronounced degree of difference is very evident on comparing the average diameters at 25 weeks under the different daily light exposures. (See fig. 3.) At 27 weeks all had developed bulbs and the tops of 4 had fallen over. Their condition in the twenty-ninth week is shown in Figure 7, B. At 31 weeks all tops had died. In the thirty-ninth week 1 plant had terminated the rest period and resumed growth. Prior to the forty-fifth week two plants had rotted. Of the remaining 3, the outer leaf scales had sloughed off, leaving in place of the old bulbs "spring" onions with new leaf growth.

Of 81 plants of this variety, only 3 showed compound formation or splitting, 1 under the 11-hour, another under the normal, and the third under the 15-hour daily light exposure. Here again splitting was not shown to be correlated with the length of daily light exposure.

SILVER KING (GIANT WHITE TRIPOLI)

Plants of the Silver King (Giant White Tripoli) variety grown under a 10-hour daily light exposure were still in the spring-onion stage at 60 weeks after planting, never having formed bulbs. Similarly, plants grown under an 11-hour exposure for 25, 30, and nearly 61 weeks remained in the condition of spring onions.

Three lots were grown under a normal light exposure. Of those planted December 12 and removed June 5, 25 weeks after planting, one had formed a good bulb which had rotted prior to removal. Although the others showed more basal swelling of the pseudostems than those in the lot grown under an 11-hour daily light exposure, they had progressed little, if any, beyond the spring-onion stage of development. Those planted November 14 on removal June 12, 30 weeks after planting, were all in the spring-onion stage but showed some basal swelling. Of those planted October 17 all were in the spring-onion stage with tops green, upright, and turgid at 35 weeks after planting, June 19. Disease entered a little later and eliminated this lot.

Though development was not uniform under a 13½-hour daily light exposure, the effect of the longer exposure on bulb formation was very evident. In the lot of 5 planted December 12 and removed at 25 weeks, 2 were unmodified spring onions, and the other 3 had developed a total of 5 bulbs. One bulb was well formed, 2 bulbs were moderately well formed, and 2 were a little more than half-formed, all with tops over. (Fig. 8, A.) Their diameter ranged from 5 to 7.6 cm. Of the lot planted November 14 and removed at 30 weeks after planting, 3 were spring onions 2 of which were compound, and a fourth had a much swollen base, whereas the remaining plant had developed a well-formed bulb with top over. Only 2 plants of those planted October 17 survived. As early as 25 weeks after planting 1 showed itself to be compounded of 2 individuals. At 40 weeks 3 bulbs had formed, measuring 6.1, 6.8, and 9.8 cm. in diameter. The top of 1 was over and that of another was dead. The first appearance of new growth was in the forty-first week. On removal at more than 60 weeks after planting, there were 3 plants in vigorous leaf growth, 1 bulb, and 2 spring onions, which had arisen from the old bulbs.

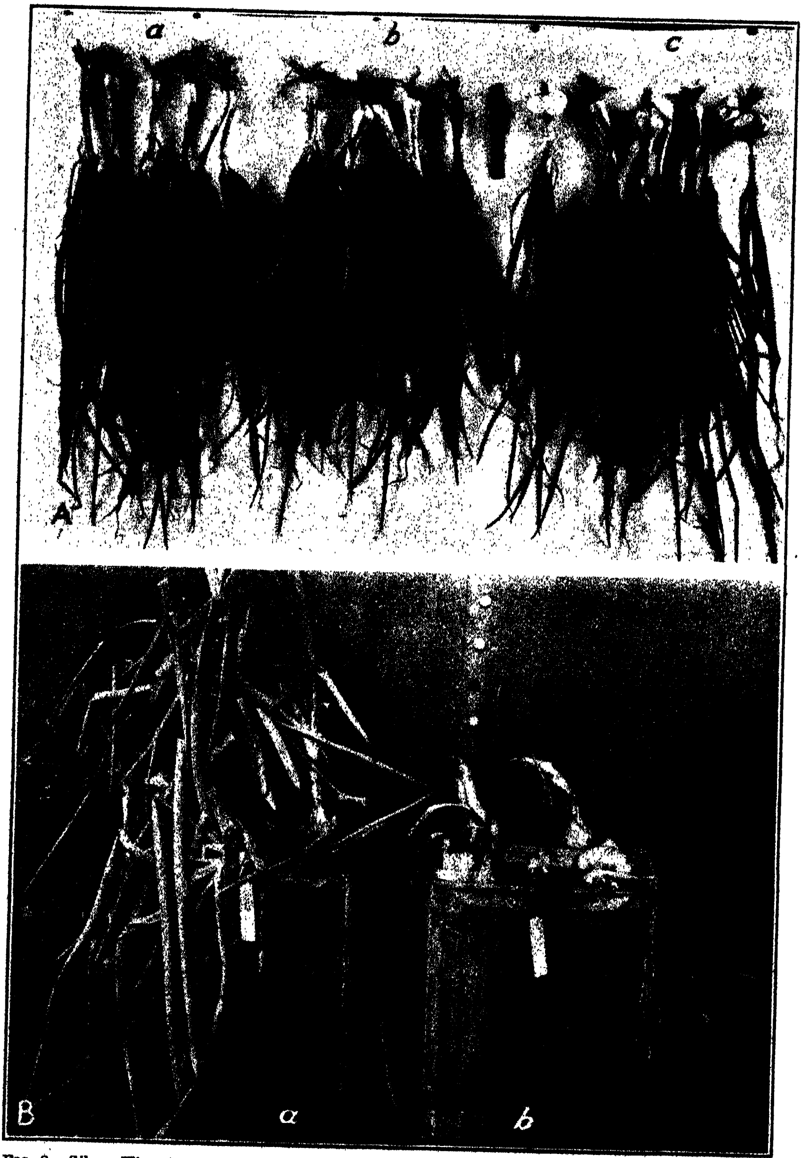

FIG. 8.—Silver King (Giant White Tripoli) onions. A, Light exposures of *a*, 11 hours; *b*, normal; and *c*, 13½ hours. December 12 to June 5. B, Light exposures of *a*, 10, and *b*, 15 hours. January 7 to July 26

Under a 15-hour daily light exposure, 2 of 5 plants showed bulbs half-developed at 20 weeks. At 22 weeks the tops of these 2 were over. At 25 weeks the tops of 2 more were over. At 27 weeks all had formed bulbs and the tops of 3 had died. Shortly after, new leaf growth started from 2 bulbs. Figure 8, B, shows their condition in the twenty-ninth week. At 35 weeks, 6 of the 8 bulbs which had developed from the 5 original plants had vigorously growing new tops. At 55 weeks, 2 bulbs had rotted, but 14 individuals, 2 of which were compound, had arisen from the other 6. All were at this time in the spring-onion stage.

As was the case with the varieties which were previously discussed, no correlation between splitting and length of daily light exposure was seen. Some splitting occurred in every planting under every exposure.

ONION SETS

The four onion varieties were grown in garden plots under normal light exposure and under a 15-hour daily light exposure. Seeds were planted February 10 in flats under normal light exposure and the resulting seedlings were set in the two groups April 4 and 5. They were removed June 3. During the time the seedlings were in the field the hours between sunrise and sunset ranged from 12.3 to 13.2. The size attained by the onions was small, the development being that of sets rather than anything larger, but the form stood in interesting relation to the daily light period. This is shown in Table 3.

TABLE 3.—*Effect of normal (12.3 to 13.2 hours) and 15-hour daily light exposures on onions grown in a garden for two months*

Variety of onion and length of daily light exposure	Number of plants	Percentage of total number of plants with				Ratio of weight of bulb or basal 3 cm. of pseudostem to weight of growth above
		Tops green			Tops dead or dying	
		Pseudostems slender	Pseudostems with intermediate swelling	Bulbs	Bulbs	
Prizetaker:						
15 hours	85	89	5	6	0	1 : 3.01
Normal	166	100	0	0	0	1 : 4.22
Bermuda White:						
15 hours	90	1	0	10	89	1 : 0.02
Normal	119	7	10	34	49	1 : 0.28
Yellow Globe Danvers:						
15 hours	75	79	13	5	3	1 : 2.41
Normal	118	100	0	0	0	1 : 4.56
Silver King (Giant White Tripoli):						
15 hours	62	57	8	16	19	1 : 1.49
Normal	87	84	11	5	0	1 : 2.69

In all the varieties the growth of tops was proportionally much greater under the normal than under the longer light exposure. The tendency toward bulb development was greater under the longer exposure, two varieties showing no tendency in this direction under the normal exposure.

POTATOES

The potato as a crop is not generally grown in Porto Rico. Occasional plantings in the hills are reported as highly successful, but the coastal plain plantings of which the writer has been cognizant have resulted mainly in crop failures.

Three varieties of potatoes, Irish Cobbler, Red Bliss, and Lookout Mountain, were planted in containers under daily light exposures of 10 and 15 hours, and in the garden. Various interfering factors eliminated the garden check. However, within three days of planting the lighted garden plot, the rows were continued for a distance of 21 to 23 feet beyond, but were not screened from the lighted plot. This continuation of the rows, not originally intended as a check, became the only check available.

The potatoes were planted in the containers January 7 and in the garden January 5 to 8. Whole tubers selected for uniformity were used, except in the continuation rows. As one or more tubers of Irish Cobbler and Lookout Mountain had failed to sprout in the containers, the tubers of these varieties were replaced at one month after planting by sprouting tubers of the same from the garden.

The Irish Cobbler plants in the containers under the 10 and the 15 hour exposures showed little difference in height up to March 12, measuring, respectively, 17 and 18 inches. Further stem elongation soon ceased under the shorter, but continued under the longer light exposure, the plants measuring, respectively, 18 and 29 inches in height April 16.

The Red Bliss, as early as seven weeks after planting, was much taller under the 15-hour than under the 10-hour exposure. The ultimate heights attained were 16 and 30 inches under the short and long exposures, respectively. The Lookout Mountain plants which February 19 measured 1 and 3 inches in height, April 16 measured 19 and 60 inches in height, under the short and long exposures, respectively. These plants were photographed April 9. (Fig. 9, A.) Buds were noted on both plants March 8, and the first blossom opened March 16 to 19.

The plants were removed April 22. Whereas all tops were green and vigorous under the longer light exposure, those of Irish Cobbler and Red Bliss had died under the shorter exposure. At this time the weight of tops under the shorter exposure was only one-sixth to one-eleventh of that under the longer exposure. The Red Bliss showed little difference in tuber formation under the two exposures, Irish Cobbler showed some difference, and Lookout Mountain a pronounced difference. The ratio of the weight of the Lookout Mountain tubers, exclusive of seed tuber, under the shorter exposure to the weight under the long exposure was as 7 to 1. (Fig. 9, B.) While the ratio of new tubers to tops was as 2 to 1 under the 10-hour exposure, it was as 1 to 23 under the 15-hour exposure. Table 4 shows the weights of tops and tubers on removal, exclusive of the tuber planted.

TABLE 4.—*Weights of tops and tubers of three varieties of potatoes which were grown under daily light exposures of 10 and 15 hours' duration*

Length of daily light exposure	Weight of Irish Cobbler		Weight of Red Bliss		Weight of Lookout Mountain	
	Tops	Tubers	Tops	Tubers	Tops	Tubers
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
10 hours	39	243	23	309	78	158
15 hours	237	190	252	321	506	22

The potatoes grown in the garden were dug April 23 to May 4. Twenty-one to twenty-six plants of each variety were grown under the lights. Although there was no satisfactory check plot, the potatoes that were grown in the extension of the rows outside the area overhung by the electric lights bore a very interesting relation to their position with respect to the lights. The rows, extending to a distance of 21 to 23 feet outside the lighted plot, were trisected, and plants nearest, midway distant, and farthest from the lights were weighed separately, 6 to 14 plants of each variety in each outside subdivision. Of each variety the plants which were grown under the lights showed the greatest leaf growth and the least tuberization. As was true for the plants grown in the containers, the differences within the variety were greatest in the case of Lookout Mountain, and least

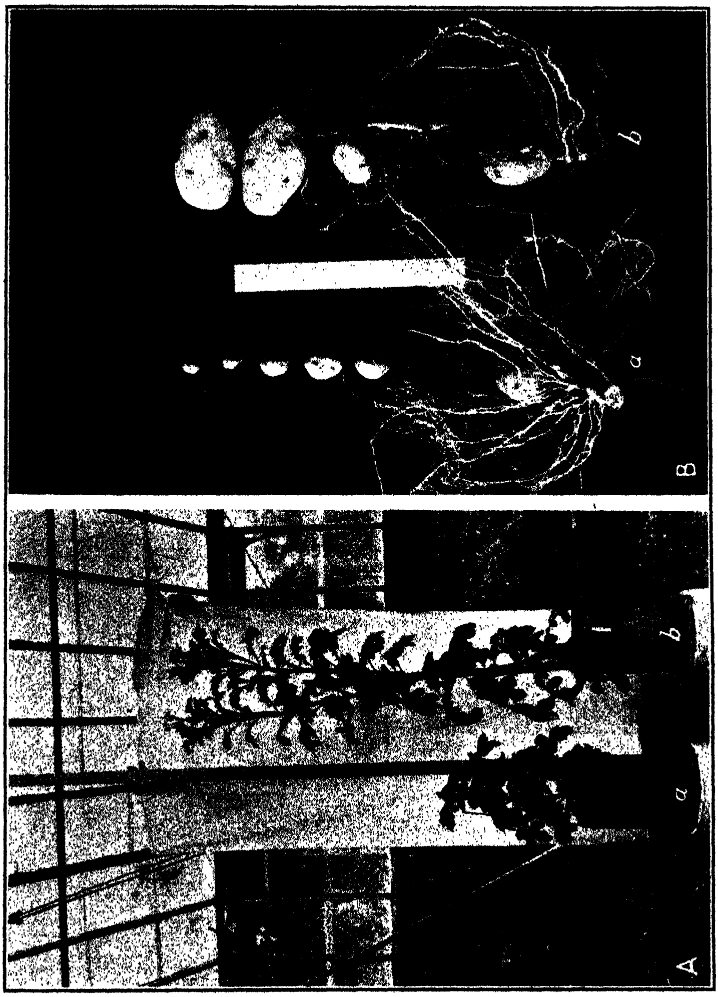

FIG. 9.—A, Lookout Mountain potatoes grown under daily light exposure of *a*, 10, and *b*, 15 hours; January 7 to April 9. B, *a* and *b*, Underground development of the potato plants shown in A: removed from the ground April 22

in the case of Red Bliss. The plants of the Lookout Mountain and Irish Cobbler varieties outside the lighted area showed a tuberization correlated with their degree of proximity to the lights, those farthest removed showing heaviest tuberization.

Table 5 gives the average weight of potato tubers per plant from plants grown under a daily light exposure of 15 hours, and from plants grown in the same rows extended 21 to 23 feet beyond the area overhung by the lights.

TABLE 5.—*Average weight of potato tubers per plant from plants which were grown under a daily light exposure of 15 hours, and from plants which were grown in the same rows extended 21 to 23 feet outside the lighted area*

Location of plants	Average weight per plant of—		
	Lookout Mountain tubers	Irish Cobbler tubers	Red Bliss tubers
	Ounces	*Ounces*	*Ounces*
Within the lighted area	0.5	2.9	5.8
Outside of, but nearest to, the lighted area	1.9	3.4	7.5
Outside of, and farther removed from, the lighted area	3.1	4.8	7.0
Outside of, and farthest removed from, the lighted area	4.8	5.1	7.1

Of 24 plants of Lookout Mountain that were grown under the lights, 11 produced no tubers, and 5 produced tubers only about the size of marbles, or smaller; of 12 plants that were grown in the outside section adjacent to the lights, 3 produced no tubers, and 3 produced tubers only about as large as marbles; of 20 plants in the two sections midway distant and farthest removed from the lights, a single plant produced tubers only the size of marbles, and the other 19 plants produced 1 or more tubers of fair size. Similarly with Irish Cobbler, but in less pronounced degree, of 21 lighted plants, 3 produced no tubers, and 1 produced tubers only as large as marbles; in the adjacent section, 2 of 12 plants produced no tubers; whereas each of 23 plants in the two remaining sections produced 1 or more tubers of fair size.

CORN

Seed of Porto Rican corn, typical of that grown in the Yauco district, was planted January 26, 1927, in the garden plots under normal and 15-hour light exposures. In each plot two rows were planted 30 inches apart, and the plats were later thinned to about 12 inches apart in the row, the plot which received added illumination containing 44 plants, and the check plot 52 plants.

On March 12 the plants under the longer light exposure stood about 3 feet high and the check plants 2½ to 3 feet high. A week later the height from the ground to the highest leaf on any individual measured 68 inches in each group. On March 26 the lighted plants on the whole stood taller than the check. From this time on the difference in height of the two groups became increasingly pronounced. Their development on April 12, 76 days after planting, is shown in Figure 10, A and B. The average height of the lighted plants was 132 inches, and that of the check was 98 inches May 13 and 14, a few days prior to the removal of the check. The seven tallest plants in each group averaged in height 158 and 121 inches, respectively.

The number of nodes per plant was counted in an entire row in each plot—half the plants. Under the lengthened light period an

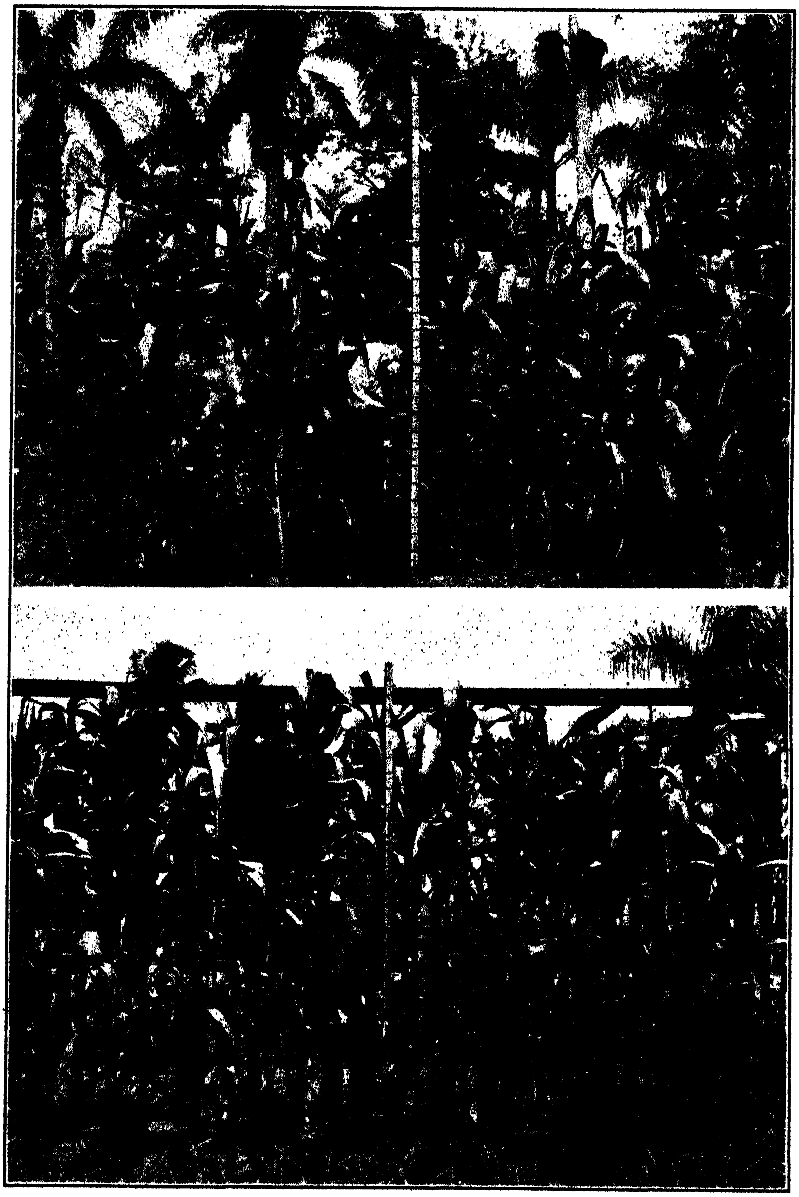

FIG. 10.—A, Porto Rican corn. Normal light exposure (11.3 to 12.5 hours); January 26 to April 12. B, Daily light exposure of 15 hours; January 26 to April 12

average of 21 nodes per plant was produced, whereas under the normal light exposure but 13.1 nodes developed. A count was made of the number of nodes, visible without disturbing the soil, from which roots had started. The average was 3.6 nodes for the lighted plants, and only 1.2 nodes for the check. In the former group, only 1 plant failed to have a single node showing roots, whereas in the latter group 11 such plants were found.

On removal, the stalks were cut at the surface of the soil and weighed after the ears were taken off. The average weight per plant of stalk and leaves from the lighted plot was 2.6 pounds, whereas that from the check was only 1.1 pounds.

Differences in fruiting, as well as in vegetative development were very marked between the two groups. Staminate flowers were first noted on the check plants on March 26, when 5 tassels appeared, and the first silks in the same group were seen two days later. By March 31, more than half the check plants had tasseled and a quarter were with silks exposed. The lighted plants when examined April 11 as yet showed no tassels, but on the day following four were showing. At this time all the normally developed plants in the check, 48 of a total of 52, had developed tassels, and 46 of these had normal ears and were silking. By April 18, only 13 of the 44 plants receiving added illumination had developed tassels, a stage which had been reached by the check plants approximately three weeks earlier.

As the two rows of corn in the electrically lighted plot stood under the outer row of electric lights, the inner corn row received a stronger illumination than did the outer row. On April 23, 17 of 21 plants had tasseled in the outer row, whereas only 10 of 23 had tasseled in the better-illuminated row. Of the 21 outer-row plants, all but 3 showed either silks or small ears, whereas of the inner-row plants a single small ear without silks exposed was as yet evident. Examined again May 3, more than one-third of the plants in the better-illuminated row showed no indication of ears forming. This row was at this time not as advanced in the reproductive stage as had been the more dimly illuminated row 10 days previously. All but 4 plants had at least rudimentary ears forming by May 14.

The height at which the ears were produced on the stalks of the lighted plants was very noticeable. Whereas in the check the number of nodes to the lowest developed ear averaged 6.4, the average for the lighted plants was 11.8 nodes. The relative position of the ear in respect to the total number of nodes produced, however, did not differ greatly between the two groups.

The corn under the normal light exposure was harvested May 19, 113 days after planting. That grown under the lengthened light exposure was harvested June 8, 20 days later, the lighting having been discontinued June 3. The lighted corn was left in the field for this additional time in order to approximate as closely as possible in the two lots the same stage of maturity at removal. The ears in the husks were weighed immediately, those from the 52 check plants weighing 36 pounds 12 ounces, and those from the 44 lighted plants 25 pounds 13 ounces, which was an average weight per plant of 11 and 9 ounces, respectively. The ratio of the weight of ears and husks to that of stalks and leaves was as 1 to 1.6 for the check plants, and 1 to 4.5 for the lighted plants.

At 35 and 36 days after harvesting, the well-dried ears were graded as uniformly as possible and weighed. As the check plot was somewhat larger than the lighted plot the data were averaged per plant. Table 6 gives the production of Porto Rican corn under normal and 15-hour daily light exposures.

TABLE 6.—*Production of Porto Rican corn under normal and 15-hour daily light exposures*

Classification of ears	Average measurements per ear				Average production per plant			
	Length under normal daily light exposure	Diameter under normal daily light exposure	Length under 15-hour exposure	Diameter under 15-hour exposure	Number of ears under normal daily light exposure	Weight under normal daily light exposure	Number of ears under 15-hour exposure	Weight under 15-hour exposure
	Inches	*Inches*	*Inches*	*Inches*		*Ounces*		*Ounces*
First grade, largest and best	8.3	1.9	8.0	1.8	0.38	3.1	0.25	1.6
Second grade, well-filled, second best	7.2	1.8	7.1	1.6	.29	1.4	.14	.5
Third grade, short well-filled to longer mostly well-filled	6.3	1.6	6.3	1.5	.17	.7	.30	.9
Fourth grade, shorter well-filled to longer poorly filled	4.9	1.3	4.7	1.2	.19	.2	.16	.2
Total					1.03	5.4	.85	3.2
Fifth grade, cobs with only a few well-developed seeds					.15	.1	.27	.1
Sixth grade, short cobs with only a few poorly developed seeds					.23	.02	.14	.02
Seventh grade, rudimentary ears and cobs without any developed seeds					.73	.02	1.00	.05
Husks						.9		.7

The measurements in Table 6 show that the production of first-grade ears by the average plant was greater under normal light exposure than under a 15-hour exposure. Moreover, the first-grade ears, though more in number, were also longer and broader under the normal exposure. The same was true in the case of the second-best ears. The combined weight of the two grades under the normal light exposure was more than twice that under the 15-hour exposure. The total production also was considerably greater under normal light exposure than with the added hours of illumination. Whereas the longer light exposure favored vegetative development, the shorter exposure favored fruiting.

PINEAPPLES

Pineapples of the Red Spanish variety were placed August 8, 1925, under the 11-hour, normal, and 13½-hour daily light exposures. Thirty vigorous slips, weighing after stripping 140 to 206 gm. each, after being placed in sequence as to weight were divided into three groups by selecting alternating plants. The groups thus formed varied in weight of average plant by less than 4 gm. The 5-gallon tin containers were filled with river gravel to within an inch of the top, the larger stones having been previously removed from the gravel by screening. Suitable fertilizer in solution was supplied uniformly to all containers, first at weekly and later at longer intervals.

The lights were as for the miscellaneous plants until May, 1927, when an additional lamp was supplied to the pineapples, owing to the shading of the lower leaves by the leaves above.

Insect damage in the heart of two plants, one in the check and the other in the long-day group, necessitated their exclusion from the comparative data.

Measurements of the longest leaf on each plant showed an average difference of 1 inch at 6 months. The range in length of longest leaf per plant at 9 months was 43½ to 53 inches, but the averages for the three groups were 48 to 49.6 inches, thus showing no material difference.

The first indications of the approach of blossoming were noted in May in the short and the normal day groups. The first plant to open flowers in the normal-day group blossomed May 22 and the second plant June 2. In the short-day group, two plants opened their first flowers June 15. No indication of blossoming was noted on plants under the longer daily light exposure until July 7. The first blossom in this group opened July 23. Prior to this time five plants in the short-day group and two in the normal-day group had finished flowering.

The fruits were picked as soon as they were considered to be fully ripe. In September, two in the short-day and two in the normal-day groups were picked, and in the short-day group three were picked in October, whereas in the long-day group the first fruit was picked November 1. The uniform fruiting and the vigorous condition of the plants of the latter group are shown in Figure 11, A, made shortly before the first fruit was picked. Whereas for the short-day group the mean fruiting date was October 24, for the check it was November 19, 26 days later, and for the long-day group December 1, 38 days later than for the short-day group. The difference between the groups in length of interim from the opening of the first blossom on a plant to the picking of the fruit was not pronounced, the season of blossoming being the main factor in determining the season of ripening. The data on production under the different length light exposures are given in Table 7.

TABLE 7.—*Red Spanish pineapple production under daily light exposures of different lengths*

Crop and length of daily light exposure	Mean ripening date [a]	Difference in mean ripening date of contemporary group receiving shortest daily light exposure and those receiving longer daily light exposures	Average length of time from blossoming to picking	Weight of average fruit	Weight expressed in percentage of weight of average fruit grown under shortest contemporary light exposure	Length of average fruit	Breadth of average fruit	Height of crown	Average number of crown slips per fruit	Average number of slips produced at base of fruit
From original plants:		*Days*	*Days*	*Grams*	*P. ct.*	*Inches*	*Inches*	*Inches*		
11 hours	Oct. 24, 1926	--------	96	1,605	100	5.8	5.2	10.0	0.4	1.8
Normal	Nov. 19, 1926	26	93	1,660	103	6.2	5.3	8.8	.8	2.3
13½ hours	Dec. 1, 1926	38	99	1,990	124	6.6	5.7	9.5	.2	4.3
From suckers:										
10 hours	Sept. 12, 1927	--------	100	1,085	100	5.1	4.8	8.6	0	.3
Normal	Oct. 3, 1927	21	100	1,177	108	5.2	4.9	9.4	0	.7
15 hours	Nov. 9, 1927	58	105	1,340	124	5.4	5.1	9.6	0	1.5

[a] Because of mechanical injuries, 1 or 2 fruits in each group of the second crop were picked prior to the normal ripening date. For each of these the normal ripening date, in order to obtain the mean, was calculated from its date of blossoming and the average number of days between blossoming and picking for the others of its group. One of these fruits had to be excluded in obtaining average weight and dimensions because of injury when immature.

Under the longer light exposure the fruits were both longer and broader, averaging 24 per cent heavier than under the short exposure, the check fruits being intermediate in size. Six of nine fruits produced under the long-light exposure surpassed in weight the largest fruit produced in either of the other groups.

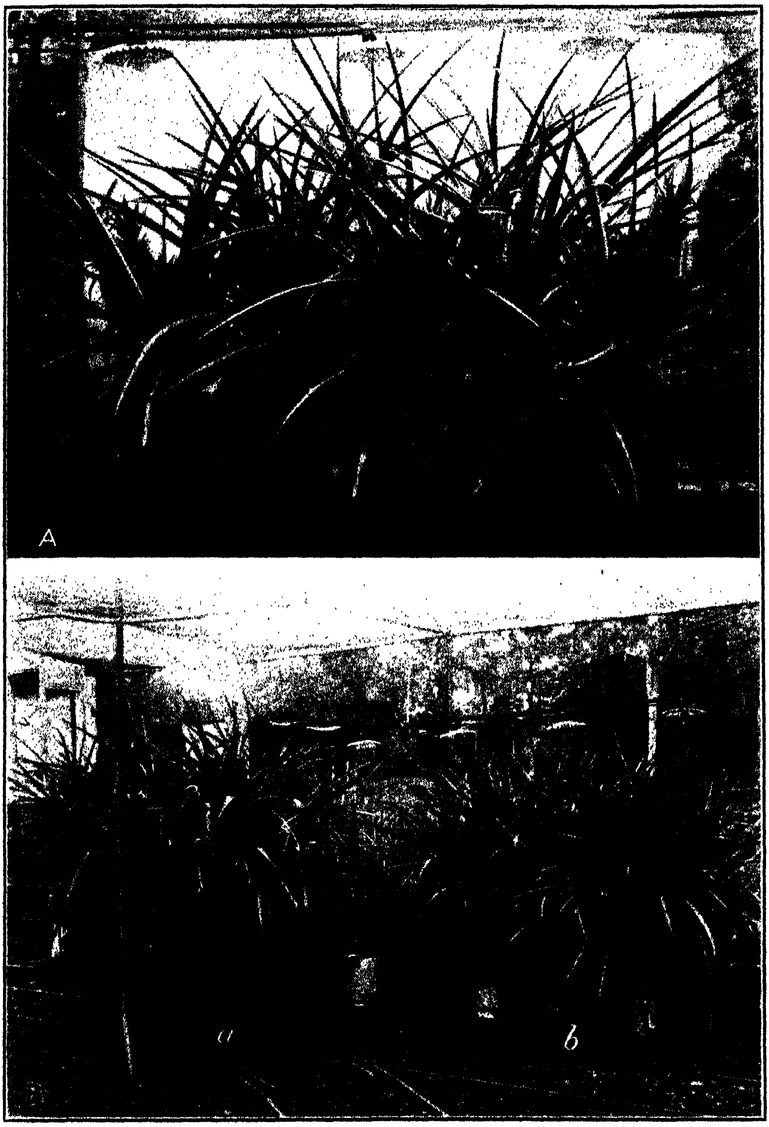

FIG. 11.—Red Spanish pineapples. A, Daily light exposure of 13½ hours; August 8, 1925, to October 30, 1926. B, Light exposures of *a*, 10, and *b*, 15 hours

The difference in the number of slips produced was also noticeable. Whereas 4.5 slips developed per fruit under the long daily light exposure, only 2.2 slips per fruit developed under the short exposure. As was the case in the size of fruit, the production of slips under the normal daily light exposure was intermediate between that of the other two groups.

In December a decided contrast was noted in the appearance of the plants in the different groups. Whereas in the normal and the short-day groups the lower leaves were yellowing, drooping, and dying, similarly located leaves in the long-day group were in the main green and turgid. And again later from time to time the much greener condition of the old pines under the long exposure was observed.

A change in the lighting period was made December 15, 1926. Although at this time two fruits remained to be picked in the long-day group, their development was too far advanced to have been materially affected by the change. Beginning at this date the lights were turned off at 15 hours after sunrise, and the plants in the short-day group were removed from the dark room at 10 hours before sunset, thus increasing the lighted period for the long-day group by 1½ hours, and shortening it for the short-day group by 1 hour. All slips were removed from the plants which had fruited, and the remaining slips were removed on picking the two remaining fruits. In each group one plant had developed two suckers, and the remaining plants one sucker each. These were left untouched.

The crop from suckers was produced considerably earlier than that from the original planting. The differences between groups in ripening season were more pronounced. The mean ripening date for the normal day group was 21 days later, and for the long-day group 58 days later than for the short-day group. (See Table 7.) The differences in stage of development June 10, 1927, are shown in Figure 11, B. The large fruits and the drooping, shrunken older leaves in the short-day group contrast strongly with the turgid older leaves and the almost total absence of fruits in the long-day group. One of the latter had blossomed at this time and three were showing buds, thus as a group just entering the blossoming stage. As was the case in the preceding crop, the difference in dates of ripening was due mainly to a difference in the blossoming dates rather than a pronounced difference in time required to mature, though this averaged slightly longer under the longer daily light exposure.

The blossoming of the group as a whole, and consequently the ripening season, of both the original and sucker plants was spread over a longer period under the normal light exposure than under either the short or long daily light exposures.

The fruits produced by the suckers, more poorly conditioned than the original plants, were smaller, but bore the same group relations. Seven of the 10 fruits produced under the long exposure surpassed in weight the largest fruit produced under the short exposure. An eighth fruit which weighed only 6 gm. less than the latter would doubtless have weighed much more had not the sucker on which it was borne fallen over more than two months prior to the ripening of the fruit, half breaking through at the base. The average fruit produced under the 15-hour daily light exposure weighed 24 per cent more than that produced under the 10-hour daily light exposure, was both longer and broader, and was subtended by a greater number of slips. Only a single fruit produced under the normal light exposure attained the weight of the average fruit produced under the 15-hour daily light exposures.

On March 7, 1928, the plants were cut at the surface of the soil, and the growth above ground was weighed. The average weight per container was 6.8 pounds under the 10-hour exposure, 7.7 pounds under the normal exposure, and 8.2 pounds under the 15-hour daily light exposure, the latter being 21 per cent greater than that under the 10-hour exposure. The weights under the 10-hour and normal exposures included some immature fruit without which the averages would have been reduced by 0.1 pound and 0.3 pound, respectively. Whereas on removal of the plants, 5 under the 10-hour exposure, and 6 under the normal exposure, carried buds, flowers, or young fruit, only 1 under the 15-hour daily light exposure had as yet budded, thus in the third crop showing retarded flowering under the lengthened period of illumination.

BEANS

Three varieties of beans were grown, two of which are the most common locally, a white and a red, the latter a plump mottled bean sometimes designated Guayamera, and a continental variety, Red Valentine. These will be referred to as White, Red, and Valentine.

From March 11, 1924, to June 30, 1925, 18 plantings were made at intervals of four weeks and later 2 additional plantings were made. The containers used were 5-gallon tin cans, each carrying river loam in amount equivalent to 50 pounds of moisture-free soil which previously was screened and well mixed. Owing to the unsatisfactory growth made in the first 9 plantings, fertilizer in suitable amount and quality was supplied thereafter. In the first 7 plantings 10 plants were grown in each can, and thereafter only 5 plants. Nematodes, insects, and diseases proved to be troublesome at times, and together with atmospheric variations caused widely different growth and production in plantings made on different dates. In some instances one or another of these disturbing factors interfered with normal growth to such an extent as to make it advisable to omit certain plantings in obtaining the averages presented in Table 8.

TABLE 8.—*Growth, blossoming, and production of three bean varieties under 11-hour, normal, and 13½-hour daily light exposures*

Variety and length of daily light exposure	Interval between planting and blossoming	Interval between blossoming and ripening of crop	Interval between planting and death of vines	Height of plant	Number of pods per plant	Weight of dry seed per plant
White:	*Days*	*Days*	*Days*	*Inches*		*Grams*
11 hours	40	32	80	19.0	3.4	2.4
Normal	41	31	84	19.8	4.1	2.8
13½ hours	42	32	90	27.6	3.9	2.9
Red:						
11 hours	30	32	77	10.5	2.3	1.3
Normal	30	32	77	9.9	2.4	1.4
13½ hours	30	32	84	12.3	2.1	1.2
Valentine:						
11 hours	33	35	67	11.7	2.5	1.2
Normal	33	35	71	10.6	3.0	1.2
13½ hours	32	34	72	13.6	2.4	1.0

The day on which the second plant to blossom opened its first flower was considered as the blossoming date for each group. No pronounced effect in hastening or retarding blossoming resulted from the difference in length of daily light exposure. In 18 plantings of the White bean the long-day group blossomed later than the short-day group in 11, contemporaneously with it in 2, and ahead of it in 5 plantings. On the whole, the long-day group averaged 2 days later than the short-day group in coming into blossom. On the other hand, the Valentine bean under the long daily light exposure blossomed on an average 1 day earlier than in the other two groups. The average of 20 plantings of the Red bean was the same under each of the three different light exposures. The extremes were 27 and 33 days and occurred in both the short and the long-day groups.

The interval between the date of blossoming and the date of ripening of the first pods on two plants was considered as representative of the length of time required for maturing pods. There was not evident in any variety any material difference in time required for ripening under the different daily light exposures as averaged from data on 12 or more plantings of each.

The relation between yield and length of daily light exposure was too inconstant to furnish conclusive evidence. In 19 plantings of the White bean, the long-day group surpassed in weight of crop the normal-day group 11 times and the short-day group 13 times. The difference in yield of plantings made on different dates but given the same daily light exposure was often greater than that of groups receiving different light exposures but planted contemporaneously. The production curves of the White bean under the three different light exposures follow the same general trend and show that other factors affected production to a greater extent than did the length of daily light exposure, veiling this effect in large measure. Whereas the White bean was on the whole less productive under the short daily light exposures, the Red and Valentine beans were slightly less productive under the long exposure. In average weight of dry seed per plant, the Red bean under the short exposure surpassed that under the long exposure in 13, equaled it in 1, and fell below it in 6 plantings.

While the height curves show in like manner a wide variation between plantings made on different dates, the favorable effect of long daily light exposures remains clearly evident. (Fig. 12.)

The height of each plant was recorded. In 18 of 20 plantings of the White bean the group receiving the longer exposure surpassed both other groups in height. Of the 20 plantings, as a whole, the average plant in the long-day group measured 39 per cent taller than in the check, and 45 per cent taller than in the short-day group. A comparison of the long and the short day groups of the Red bean showed that while the difference in height was less pronounced, the effect of the longer daily illumination was no less evident than in the preceding variety. In only a single one of the 20 plantings was the long-day group surpassed in height by either the short-day group or the check. In 15 plantings of Valentine beans, the long-day group surpassed the check in height in every planting and the short-day group in 10 plantings.

The length of life of the plant was reckoned from the date of planting to the date on which the plant neither carried live leaves nor was putting out new growth. The individual life span was recorded in all plantings after the seventh. The long-day group exceeded in average length of life the normal-day group in 9, and the short-day group in 8, of 13 plantings of White beans. The average length of life under the short, normal, and long daily light exposures was 81, 84, and 91 days, respectively. Similarly, the average length of life of the Red bean was 84 days under the long daily light exposure, and 77 days in the other two groups; and that of the Valentine bean was 67, 71, and 72 days for the short, normal, and long day groups, respectively.

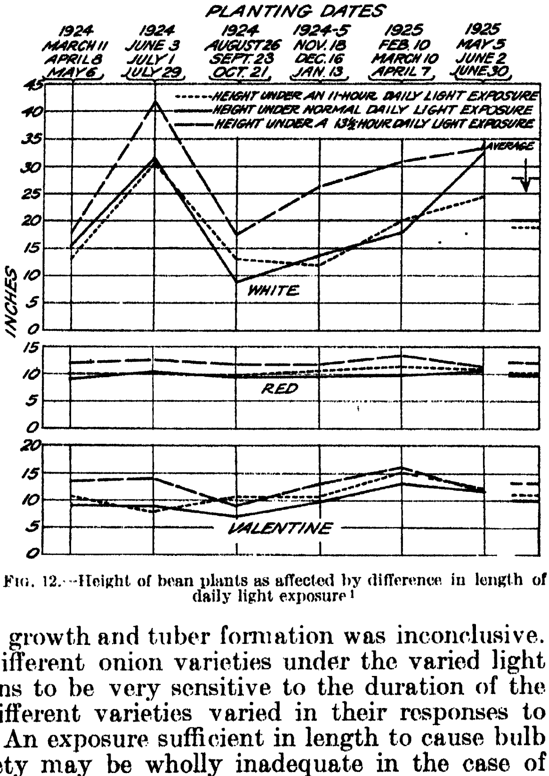

FIG. 12.—Height of bean plants as affected by difference in length of daily light exposure[1]

DISCUSSION AND SUMMARY

Economic plants of different varieties were grown under daily light exposures of 10, 11, 13½, and 15 hours, and under the normal light exposure, 11 to 13.2 hours (sunrise to sunset).

An 11-hour daily light exposure proved to be more favorable to the blossoming of both Porto Rico and Key West sweet potatoes than a 13½-hour exposure. The evidence as to the effect of these light exposures on vine growth and tuber formation was inconclusive.

The growth of the different onion varieties under the varied light exposures showed onions to be very sensitive to the duration of the daily light period. Different varieties varied in their responses to different day lengths. An exposure sufficient in length to cause bulb formation in one variety may be wholly inadequate in the case of another. For this reason, certain varieties which form large bulbs in temperate latitudes may fail wholly to form bulbs when grown in the Tropics. In the lower latitudes where the temperature permits planting throughout the year, not only is it essential to select such varieties as will form bulbs under the day lengths which prevail in these latitudes, but the favorable planting time as well must be determined with regard to the seasonal day length. While the shorter days favor leaf growth, they inhibit bulb formation, for which the longer days are necessary. Of the four varieties included in these tests, one only, Bermuda White, showed itself to be well adapted for growing in such latitudes as have a maximum day length only shortly in excess of 13 hours. Though some individuals of the Silver King (Giant White Tripoli) variety formed bulbs, the varietal adaptability to such day lengths could be considered as only partial. The Prizetaker and Yellow Globe Danvers varieties are wholly unsuited for growing under such day lengths, plants in the main remaining in the spring-onion stage rather than forming bulbs. Under daily light exposures of a little more than 15 hours, normal bulb formation was rapid in all four varieties.

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[1] Each point determined by average height of 3 plantings, except Valentine second and fifth points determined by 2, and third point by 1 planting.

The tendency of the onion to form more than one core, or to split into two or more individuals was not shown to be correlated with the length of daily light exposure, but rather to be a varietal characteristic. This tendency was most pronounced in Bermuda White and Silver King (Giant White Tripoli), and least evident in Yellow Globe Danvers. Owing to vigorous top growth and absence of bulb formation under the shorter exposures, the splitting of one individual into several was more conspicuous under the shorter than under the longer daily light exposures where bulbs were quickly formed and the resting period was entered. On resumption of growth the compound formation of the bulb under the longer exposures became evident.

The Irish Cobbler, Red Bliss, and Lookout Mountain varieties of potato differed considerably in sensitiveness to differences in length of daily light exposure. Of the three the Lookout Mountain variety proved to be the most sensitive, and the Red Bliss the least so. Whereas the longer exposure favored growth of tops, the shorter exposure favored tuberization.

Porto Rican corn showed pronounced differences in growth and production under normal and 15-hour daily light exposures. Under the lengthened exposure blossoming was delayed, a much greater height was attained, and the production was inferior in number and size of ears to that under the normal light exposure.

Red Spanish pineapples were grown under normal, 10, 11, 13½, and 15-hour daily light exposures. Under the longer exposures blossoming was delayed, the size of the fruit was considerably larger, the production of slips was notably greater, and the total growth was increased.

The most clearly evident and consistent effect of the longer daily light exposure under which the beans were grown, 13½ hours, was the increased vegetative activity as shown by the greater height and greater length of life under the longer daily illumination.

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EXPERIMENTAL METHODS AND THE PROBABLE ERROR IN FIELD EXPERIMENTS WITH SORGHUM[1]

By JOSEPH C. STEPHENS, *Junior Agronomist*, with introductory statement by H. N. VINALL, *Senior Agronomist, Office of Forage Crops, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

THE FIELD STATION AT CHILLICOTHE, TEX.

In 1915 the location of the Chillicothe (Tex.) Field Station was changed from a small rented tract adjoining the town site of Chillicothe on the northwest to a larger field 4½ miles south and 1 mile west of the city. The land and buildings thereon were purchased by the Texas Agricultural Experiment Station in 1916 and formally incorporated in its organization as Texas Substation No. 12. The forage-crop experiments carried on at Chillicothe are all conducted under a cooperative agreement between the Office of Forage Crops, Bureau of Plant Industry, United States Department of Agriculture, and the Texas Agricultural Experiment Station.

Approximately 100 acres of land are included in the station boundaries, and this land is unusually level, although it has a gradual slope from the southeast to the northwest. (Fig. 1.) This natural drainage, a fall of approximately 16 feet in 188 rods, has been controlled by ditches so that it is carried almost due west directly through the experimental tract by a large ditch banked slightly on the north side. To catch the drainage water from the field south of the station, a smaller ditch has been constructed along the south border for about three-fourths the distance from the west side. The building site and pasture lots occupy about 14 acres in the southeastern corner of the station tract, and the remainder of the land has been divided into blocks 20 rods square, except for strips of land on the north, south, and west sides, as indicated in Figure 1. Separating the blocks are roadways 20 feet wide, except for the main drive. These square blocks were laid off thus to allow for a change in the direction of the rows and plots each year. Most of the experiments are with crops grown in cultivated rows, 40 inches apart, such as sorghum, cotton, and cowpeas, and the plots are approximately twelve times as long as they are wide. By having the direction of the plots north and south one year and east and west the following year, the effect of alleyways between plots is overcome. The general arrangement of the land for experimental purposes is illustrated in Figure 1, a topographical chart which also indicates the elevations at different points.

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[1] Received for publication Aug. 6, 1928; issued December, 1928.

The soil is a dark, sandy loam which from general observation appears more uniform than the average experimental tract of this size. It lies entirely on the rolling prairie at an altitude of 1,406 feet. The surface soils are derived from the Permian formation and belong mostly to the Vernon and Kirkland series. Certain small areas, the surfaces of which had been removed in leveling the land, thus bringing the calcareous strata nearer the surface, were mapped as Hardeman soils.

A somewhat detailed soil survey of the station tract was made in 1919.[2] The results of this survey are indicated in Figure 2. The experiment described in this paper was located in block G2 on the west side of the station tract, situated in 1915 as indicated by the hatched area on the soil map. In block G2 as then located only soils of the Kirkland series are found. Most of the area is occupied by Kirkland loam, the surface 6 to 8 inches of which is a dark-browy friable loam which assumes a grayish color on top when it is dry. The subsoil down to 24 inches is a dark-brown or brown friable clay. From 24 to 36 inches of the subsoil is a grayish brown slightly calcareous clay which at 36 inches is nearly yellow or has yellow spots in it.

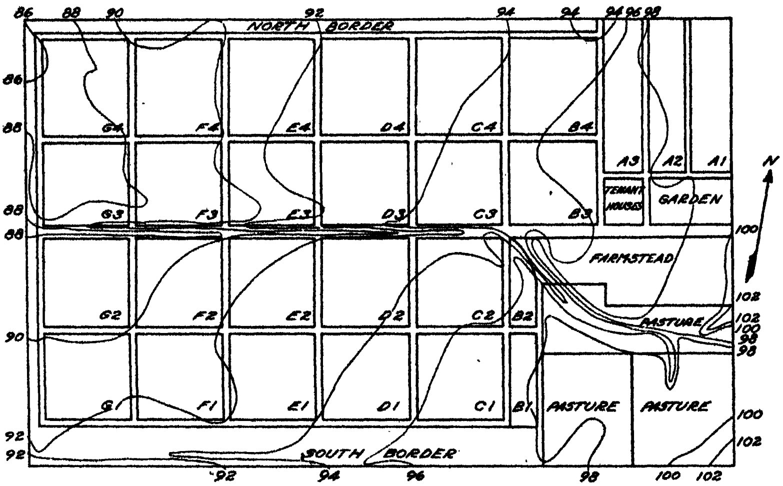

FIG. 1.—Topographical chart of Texas Substation No. 12, Chillicothe, Tex., showing contour lines and divisions of the area for experimental purposes

Kirkland clay loam differs from the Kirkland loam mainly in that the surface soil is a clay loam or silty clay loam and in some places is only 4 inches deep. The subsoil is about the same as that of the first type. Kirkland silt loam, as the name indicates, has a friable silt loam for the surface 8 inches. This surface soil grades into a silty clay loam beneath and at 16 to 20 inches becomes much more compact. At 20 inches it is grayish brown speckled with white, gray, and brown clay, and the subsoil is quite calcareous. The Kirkland fine sandy loam is found on the lower levels and is different from the loam chiefly because erosion from higher levels has left a deposit of fine sand on it, and this has been mixed with the surface 6 to 8 inches through cultivation.

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[2] This survey was carried out under the direction of W. T. Carter, Bureau of Chemistry and Soils, U. S. Department of Agriculture, for the Texas Agricultural Experiment Station, by T. M. Bushnell, A. C. Anderson, and Neal Gearreald.

The land on which the station is located had been farmed for a good many years, very largely to uniform crops of wheat, with only occasional crops of cotton. Although not so productive as some of the soils in northern Texas, this land was of at least average fertility, lacking chiefly in humus. When the land was taken over it was decided to follow a rotation consisting of small grain (wheat or oats), sorghum, cotton, and cowpeas. This had the advantage of ridding the soil of volunteer plants and (as much of the cowpea crop was plowed under) of adding a certain amount of humus to the soil. Owing to the pressure for an increased acreage of land for the cotton experiments and the small importance of the cowpea crop in this section, it has not been found possible to continue this rotation in its entirety. The results indicate, however, that the productiveness of the soil and its uniformity as a whole have increased under this method of handling the experiments. The strips of land on the north and south sides and the blocks north of the building site are used for miscellaneous experiments which do not fit into the general rotation. A tract of about 12 acres south of the building site, except for gardens and a small orchard, is devoted to pasture for the work stock and milk cows.

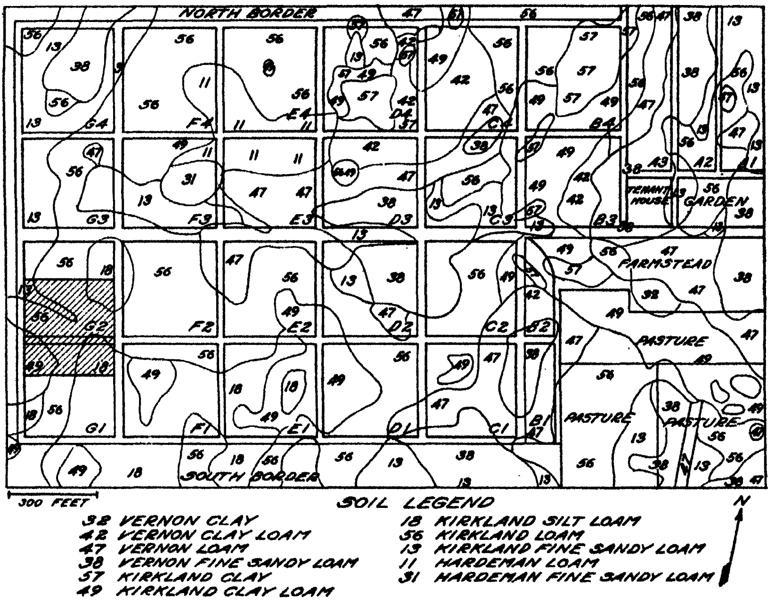

FIG. 2.—Soil map of Texas Substation No. 12, showing soil types and the location of experimental area G2 as situated in 1915

PURPOSE OF THE EXPERIMENT

The removal of the station to a new location provided an opportunity and also a reason for an experiment to determine the extent of error which might be expected on this land because of the soil heterogeneity. Since sorghum was destined to be one of the chief crops in the work at Chillicothe, it was used as the indicator crop. A very uniform strain of feterita which had been selected for six years was seeded over an entire block in rows 40 inches apart and given the same cultural treatment as that accorded to the regular experiments. In seeding this block to feterita (F. C. 811), the chief thought was to obtain an increased quantity of the seed, and it was not until later in the year, as harvest time approached, that the idea developed of using this 2½ acres of feterita for the purpose of estimating the experimental error to be expected in future tests of sorghum. No extra precautions, such as replanting portions of the rows where vacancies in stand occurred, were taken. It is recognized that in any well-conducted experiment the number of unknown factors should be limited, if possible, to one. The fact remains, however, that in ordinary field experiments very few are so handled as to exclude completely little discrepancies of this sort. The results cover, therefore, not only differences due to soil heterogeneity, but also others due to imperfections in the stand such as appear in the average field experiment plot. That such imperfections were less, or at least no greater, in this block of feterita than in the better conducted field experiments, was the judgment of several who observed it closely before it was harvested. For the purpose of measuring the error that must be taken into consideration when conducting varietal or cultural experiments on the Chillicothe station farm, the results obtained are perhaps more useful than if unusual care had been taken to eliminate all effects on the yield other than those due to soil differences.

LOCATION OF THE EXPERIMENT

This feterita was grown on block G2, in the southwest corner of the station tract. The location of this block was about 8 rods farther south than at present. (See fig. 2 for present location.) The change was necessitated by drainage requirements which altered the location of the main ditch to provide for it directly adjacent to and parallel with the main drive through the experimental field from the entrance on the east to the west boundary. This drive and the ditch together occupy a strip of land about 40 feet wide through the middle of the station tract and withhold from experimental uses approximately 2½ acres of land. No better way of handling the surface water could be devised, however, because heavy torrential rains sometimes occur, and small shallow ditches overflow and allow sand and silt to be deposited on the experimental areas. An occurrence of this kind early in 1915 destroyed the uniformity of blocks G3 and G4.

Block G2 on which the feterita was grown, while not the most uniform area on the station, was no doubt of at least average uniformity. It had grown wheat the previous year and was therefore subjected to uniform culture processes. It is exceptionally level and has no drainage course through it.

CLIMATIC FEATURES OF 1915

Although not of any great importance in an experiment of this kind, since all the plants in the block are subjected to the same climatic conditions whether they are good or bad, it is perhaps worth while to indicate briefly the general seasonal conditions.

The total annual rainfall in 1915 was 34.81 inches, 8.8 inches above a 21-year average. The total for the growing season, May to September, inclusive, was 20.5 inches, 5.6 inches more than normal. The mean temperatures for the same months were: May, 68° F.; June, 78°; July, 81°; August, 76°; September, 75°. The lack of any effective rain from June 6 to July 19 resulted in a drought during July which reduced the yields somewhat.

CULTURAL METHODS

The ground was plowed and harrowed during January and February. It was disked and marked off in rows 40 inches apart preparatory to seeding in late May. The seed was sown with a drill, and it germinated to a fair stand. Very little thinning was required, and a count of the plants showed an average of 616 plants per row, which indicated an average row space of a trifle over 6 inches to each plant. The stand was estimated to be 90 per cent uniform, with only occasional small breaks. Only ordinary cultivation was given the crop, but when mature it was comparatively free from weeds, and the soil was at all times in good condition for growth.

METHODS OF HARVESTING

At harvest time the average height of the stems was 55 inches and the diameter five-eighths inch. R. W. Edwards, then superintendent of the station, reported observations as follows: "The west side of the block was not so good as the east side. [This proved true. Compare mean yields of "east third" and "west third," Figure 6.—H. N. V.] A large number of sucker heads were formed, most of which matured." The rows were marked off in rod lengths and harvested by hand. Weights of the green forage from each separate rod were obtained immediately as it was cut. The weights presented in this paper, therefore, represent green material. The yield was approximately 4.5 tons per acre, equivalent to 1.8 tons air-dry forage, a normal yield for this dwarf and rather fine-stemmed feterita.

The intention was to incorporate the data thus obtained in a publication on experimental methods at once, and the weights were reduced to ounces and the row totals calculated in the winter of 1915–16. Other more pressing work intervened, however, and delayed the completion of the task until the services of Mr. Stephens were obtained in 1926. No attempt has been made to review other publications on this subject. None has been presented which deals with the forage yields of sorghum grown in cultivated rows, and this contribution is made with the thought that it may serve to stimulate a desire to employ more accurate methods in experiments with such crops.

FIELD EXPERIMENTS

Original yields of the feterita are given in Table 1. No new methods have been introduced in handling the data, and the procedure is subject to whatever criticism may properly be directed at the usual ways of conducting blank experiments. Several plans advanced for reducing the error in field experiments have not been applied to the data here.

TABLE 1.—*Experimental-error data in the sorghum experiment at Chillicothe, Tex., in 1915*

Row	Yield (ounces) of 1-rod portions of the rows of feterita in the order in which they occur in the field (facing east)																				Row
1	226	222	236	262	208	213	200	204	184	230	206	196	194	220	158	160	200	152	186	145	1
2	236	178	204	234	268	202	190	205	186	210	168	160	201	194	232	160	210	250	214	176	2
3	224	202	210	262	210	191	190	179	184	218	175	160	191	220	194	190	236	200	204	112	3
4	240	196	264	252	216	240	208	168	152	180	176	200	200	181	216	176	204	212	212	166	4
5	216	233	214	208	244	241	210	211	230	168	190	142	192	188	176	184	160	196	193	160	5
6	224	222	198	185	183	180	195	184	159	204	168	168	216	250	224	200	242	202	140	190	6
7	236	272	270	228	244	214	202	222	208	204	212	146	182	224	182	200	209	215	182	190	7
8	198	177	228	202	174	218	201	232	138	173	158	142	232	220	216	180	224	190	212	198	8
9	202	174	210	192	192	222	191	164	170	170	174	128	172	212	214	140	202	210	198	202	9
10	210	190	200	232	208	218	262	226	137	174	182	168	184	186	196	184	208	200	176	234	10
11	234	180	168	200	192	196	190	194	186	160	196	162	196	198	158	168	212	208	184	188	11
12	222	166	214	210	188	240	220	190	194	142	172	146	198	208	208	150	208	230	164	212	12
13	182	204	228	228	216	218	232	214	188	170	190	138	208	182	210	220	216	204	174	214	13
14	294	200	216	185	218	172	194	200	162	172	182	177	204	198	164	155	201	214	176	184	14
15	230	228	220	230	228	208	220	187	144	162	176	168	232	212	212	218	108	182	216	190	15
16	244	198	216	257	176	198	234	214	176	180	195	158	202	188	152	138	193	196	174	226	16
17	188	172	222	200	234	225	242	208	185	150	200	156	196	208	183	192	220	164	202	186	17
18	204	167	206	160	178	184	200	154	176	144	131	113	174	196	171	150	162	201	188	200	18
19	212	232	220	248	238	254	210	244	196	212	188	160	174	188	186	186	192	218	190	212	19
20	201	176	168	188	122	152	177	192	146	192	152	149	158	182	182	158	222	208	190	188	20
21	202	166	184	216	222	162	188	166	170	148	162	166	154	174	172	154	238	228	228	190	21
22	172	180	188	120	196	226	182	170	158	198	176	148	158	144	158	160	214	224	178	182	22
23	274	166	182	216	178	224	182	150	158	162	150	150	162	172	176	160	224	212	217	192	23
24	184	144	202	192	150	184	180	192	126	174	154	144	152	166	162	168	200	174	192	164	24
25	232	178	188	242	224	210	184	176	184	138	208	182	116	184	185	148	180	206	202	176	25
26	182	184	196	172	168	128	164	162	164	134	114	104	154	188	192	140	163	178	194	168	26
27	194	196	166	228	200	164	180	160	116	148	168	158	144	162	166	140	192	174	202	180	27
28	166	182	164	166	216	216	208	194	140	170	118	154	168	208	160	150	190	214	168	158	28
29	206	126	159	172	192	148	202	212	153	130	140	148	157	183	122	164	166	210	209	143	29
30	196	140	145	127	216	172	193	181	193	190	129	120	158	161	170	146	210	187	170	178	30
31	193	200	175	170	192	177	187	200	204	241	149	167	186	218	197	198	193	194	168	151	31
32	204	148	201	165	186	181	185	161	163	168	188	172	162	200	160	173	169	189	185	147	32
33	185	186	153	154	168	134	126	181	147	135	163	145	173	173	101	178	186	174	176	140	33
34	200	208	200	186	211	186	193	169	177	142	188	148	161	188	202	198	178	172	176	180	34
35	208	170	189	153	200	176	185	213	165	161	151	201	193	136	180	153	206	144	201	104	35
36	185	150	150	169	178	167	186	192	161	167	182	132	145	148	110	122	162	174	153	163	36
37	210	180	208	225	213	239	183	237	167	202	204	249	206	202	209	292	183	185	196	168	37
38	164	188	176	184	185	166	151	173	167	170	197	180	198	200	156	161	151	143	129	143	38
39	182	185	207	219	159	144	207	208	123	117	208	200	175	170	128	170	144	165	148	154	39
40	243	214	225	144	151	194	200	206	191	199	223	169	208	181	149	179	141	138	187	169	40
41	212	165	165	212	134	175	184	174	128	194	190	200	180	159	172	144	134	190	162	155	41
42	196	206	149	196	173	184	177	174	173	170	168	169	176	121	156	172	163	150	160	167	42
43	192	209	215	158	202	202	197	182	192	202	197	193	162	183	207	177	162	151	165	192	43
44	203	191	164	158	159	168	169	184	167	179	177	157	165	145	175	179	145	159	131	138	44
45	174	175	208	205	184	178	183	208	161	165	179	184	173	181	218	203	180	126	172	177	45
46	216	192	202	183	167	182	209	186	160	184	204	157	169	162	221	170	182	152	164	196	46
47	178	159	218	192	149	183	137	158	125	213	155	172	169	189	200	198	173	137	176	148	47
48	229	197	171	175	202	159	161	149	91	148	150	164	188	167	176	128	136	139	116	140	48

49	242	185	199	198	204	185	191	138	189	191	169	173	193	245	156	191	190	164	146	175	49
50	193	172	195	147	173	167	156	129	146	172	191	142	189	163	178	185	193	161	150	141	50
51	246	131	205	208	176	184	150	143	162	146	158	158	196	192	204	160	153	150	144	181	51
52	216	185	189	188	205	184	173	161	162	147	166	156	173	232	182	200	185	188	154	198	52
53	165	153	205	193	201	172	125	180	142	162	120	172	187	186	167	178	204	136	136	176	53
54	203	167	198	182	214	184	140	179	153	203	197	141	201	191	236	196	169	165	200	168	54
55	200	217	188	233	247	218	186	221	201	151	155	192	188	186	197	199	197	176	175	147	55
56	193	229	224	170	190	168	154	179	217	212	193	211	232	227	215	216	173	216	188	217	56
57	220	249	195	189	245	161	199	212	179	188	225	210	176	213	165	189	173	202	202	164	57
58	212	202	232	224	186	176	162	195	168	208	199	216	219	200	183	199	165	154	184	174	58
59	227	234	195	255	240	169	217	215	206	235	193	207	244	192	156	199	183	197	169	134	59
60	177	154	179	208	173	143	169	143	149	161	175	173	189	171	180	204	174	155	192	161	60
61	175	252	229	250	215	129	180	200	188	173	198	198	236	232	217	198	187	193	222	201	61
62	148	167	150	152	175	139	161	187	154	194	181	302	143	214	218	194	187	166	188	196	62
63	165	227	185	184	135	166	188	202	179	206	177	193	213	186	183	165	215	200	170	190	63
64	206	160	170	178	160	168	185	205	140	132	188	161	214	210	201	194	216	175	193	172	64
65	216	182	179	187	172	194	186	223	177	212	185	222	214	184	172	163	194	207	186	153	65
66	184	203	155	128	121	172	150	144	195	186	191	212	221	187	180	163	179	167	206	160	66
67	234	213	163	155	176	160	191	198	207	225	184	194	227	119	182	181	213	205	188	185	67
68	186	156	152	98	147	152	171	172	190	167	201	224	237	209	212	178	180	258	75	191	68
69	179	176	142	99	146	132	187	198	229	195	203	238	250	201	178	203	180	209	214	160	69
70	214	187	153	167	167	200	179	168	173	188	206	222	257	212	222	196	181	213	214	161	70
71	205	180	160	131	151	124	193	190	163	170	129	211	222	203	222	208	181	184	196	155	71
72	165	170	188	179	183	136	186	136	147	166	170	204	199	192	186	177	175	144	198	170	72
73	158	179	147	152	175	190	154	175	169	191	212	260	132	169	202	207	195	179	194	177	73
74	189	159	156	143	172	172	156	193	142	183	210	204	176	179	199	184	132	164	188	163	74
75	190	203	152	165	144	167	153	178	182	176	173	184	176	181	150	191	186	194	197	160	75
76	193	141	186	177	177	149	168	177	199	199	174	181	194	158	189	184	172	186	174	174	76
77	165	126	141	131	126	147	188	191	216	208	213	197	164	156	158	178	192	178	132	163	77
78	156	144	136	147	136	155	162	163	188	226	188	144	157	196	150	177	155	136	193	163	78
79	170	132	193	173	170	161	204	157	194	192	199	188	156	200	176	184	181	177	152	168	79
80	144	145	120	158	135	170	156	230	191	150	172	162	160	162	134	184	185	170	149	157	80
81	164	152	187	135	137	156	149	181	177	170	178	141	143	148	156	162	165	160	170	148	81
82	180	153	210	153	148	121	169	209	187	208	187	182	173	182	161	179	208	198	180	166	82
83	153	180	205	139	166	171	184	173	152	145	161	166	123	140	119	116	168	143	150	148	83
84	154	193	162	166	152	145	162	205	182	184	196	198	175	162	149	194	170	140	168	198	84
85	153	205	196	138	163	135	163	170	197	164	184	231	203	190	194	141	169	195	176	197	85
86	126	148	122	162	145	107	170	181	202	178	135	127	176	120	162	195	177	144	178	184	86
87	180	144	162	163	157	194	140	170	170	158	159	166	175	186	113	138	178	178	229	154	87
88	152	147	146	166	162	171	143	140	171	176	171	153	172	127	152	187	220	162	194	198	88
89	161	139	132	152	190	136	164	148	154	138	134	144	161	190	135	165	155	183	140	180	89
90	144	146	114	144	149	139	160	170	159	137	183	113	184	176	143	136	151	144	144	161	90
91	183	203	157	144	147	151	184	176	205	201	174	216	174	184	181	209	178	200	180	176	91
92	158	150	176	188	156	190	152	224	191	185	165	142	177	141	191	196	171	160	185	208	92
93	161	176	175	186	192	176	205	167	191	164	192	171	176	145	160	163	172	170	179	164	93
94	183	207	205	239	196	122	172	236	185	197	166	178	162	156	172	188	177	176	192	169	94
95	172	165	176	158	172	161	165	192	161	198	218	156	185	156	156	146	128	150	163	104	95
96	180	132	167	165	175	172	189	177	157	163	182	191	124	132	167	174	141	152	176	166	96
97	176	214	207	223	224	226	164	214	205	202	240	203	231	188	202	192	214	196	187	176	97
98	208	170	201	170	201	146	167	172	209	160	185	133	158	165	165	199	200	224	213	191	98
99	187	221	218	185	211	195	188	177	202	170	176	152	233	187	199	200	167	186	180	166	99
100	278	180	261	192	252	209	217	206	224	199	228	214	191	185	174	212	192	213	201	233	100

The unit area of 1-rod row 40 inches wide in which this block of feterita was harvested is $^{1}/_{792}$ of an acre. The ultimate or rod-row plot is called $^{1}/_{800}$ acre, and combination plots are multiples of $^{1}/_{800}$ instead of the actual fraction. The difference is insignificant, and the fractions used are easier to follow. Thus, the largest combination plot is $^{160}/_{396}$, but is is called $^{160}/_{400}$ or $^{2}/_{5}$ acre.

The block was 20 rods long and 100 rows wide, therefore there were 2,000 ultimate plots. The two outside rows on each side were discarded, which left 1,920 plots to be used in the calculations. These rows were discarded on the basis of the following border effect:

Row 1 plus 100, average yield, 14.34 per cent above mean of inside 98 rows.
Row 2 plus 99, average yield, 9.22 per cent above mean of inside 96 rows.
Row 3 plus 98, average yield, 5.33 per cent above mean of inside 94 rows.

The probable error of a single two-row plot (adjacent rows and using all rows) is 4.53 per cent of the mean. The two outside rows show probable border effect, but the third rows, while above the mean, differ from the mean only a little more than the probable error of a single two-row plot. The outside rod rows at the ends of each row (tiers 1 and 20) were not discarded, since their mean was only 1.95 per cent above the mean of the inside 18 tiers of rod rows.

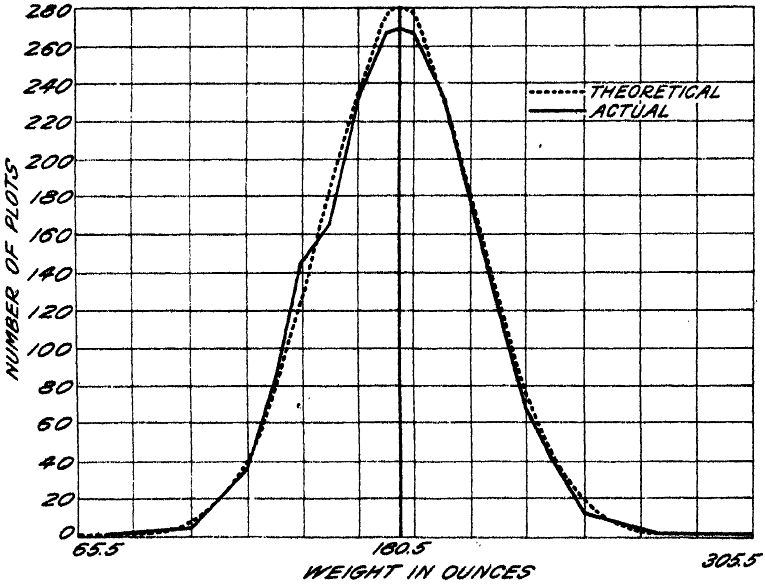

FIG. 3.—Frequency distribution of ultimate plot yields compared with normal curve of error

Ultimate plots vary in yield from 75 to 302 ounces and fluctuate about a mean of a little over 180. The distribution of these plot yields is approximately normal, as may be seen from a comparison of the actual curve and the normal curve of error[3] in Figure 3. If a grouping in the tails of the curve is used, the probability[4] that deviations as great as or greater than those observed will occur in random samplings is 0.66, or, in two cases out of three, random samples would diverge as widely as the observed from the normal curve of error.

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[3] FELDMAN, W. M. BIOMATHEMATICS, BEING THE PRINCIPLES OF MATHEMATICS FOR STUDENTS OF BIOLOGICAL SCIENCE. p. 366-370. London, Charles Griffin and Company, Ltd. 1923.

[4] ELDERTON, W. P. TABLES FOR TESTING THE GOODNESS OF FIT OF THEORY TO OBSERVATION. Biometrika 1: [155]-163. 1902.

The agreement of actual and theoretical is very close and justifies determination of the normal curve constants for the ultimate plots. It does not follow that the curves of combination plots will approach the normal as closely as did that of the ultimate plots, but it may be assumed that the combination plots are fluctations of a normal curve and that they would take the expected form if the number were large enough.

Variation is here expressed in probable error as a percentage of the mean. The probable error of a single ultimate or $\frac{1}{800}$-acre plot is 10.607 per cent of the mean.

$$y = \frac{N}{\sigma\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}}$$

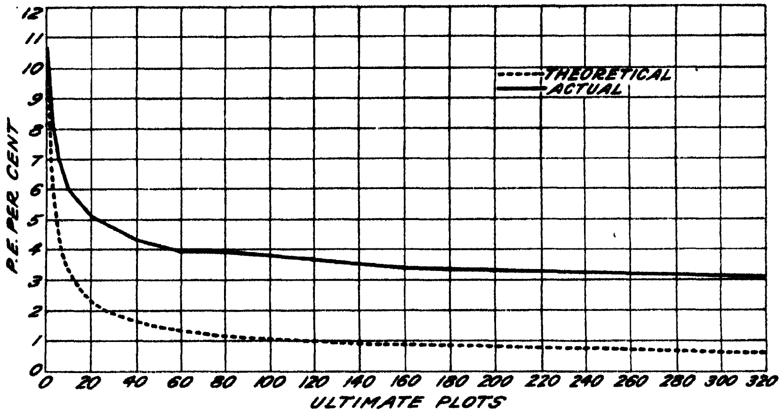

FIG. 4—Reduction in probable error due to increase in size of the plot

REDUCTION IN PROBABLE ERROR DUE TO INCREASE IN SIZE OF PLOT

The reduction in probable error due to increasing the size of the plot by grouping adjacent plots is given in Table 2 and Figure 4. Expected reduction by grouping like numbers of ultimate plots drawn at random also is shown. So far as possible in a series of increasing size, either the length or the width of the plots was kept constant and the area enlarged by addition in the other dimension.

Within the same general shape there is without exception a reduction in error due to the increase in the size of the plot. Of this method of comparing sizes Stadler[5] says:

> Increasing size of plot can hardly fail to decrease variability, for when two plots are combined the relative deviation can not be increased and is almost certain to be decreased, at least occasionally, by the canceling of plus and minus deviation. . . . But when increasing size of plot increases the size of the field, as it must if we retain the same number of plots, additional soil variations are almost always brought in, and variability of the larger plots is increased. In com-

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[5] STADLER, L. J. EXPERIMENTAL ERROR IN FIELD-PLOT TRIALS. Paper read before Internatl. Cong. Plant Sci., Ithaca, New York, Aug., 1926. [Not published. Title in program, p. 19.]

paring large and small plots on the basis of equal numbers the advantage of large plots is therefore likely to be less than that indicated by the blank experiments.

TABLE 2.—*Reduction in probable error due to increase in size of the plot*

Approximate size of plot (acre)	Shape of plot			Probable error of single plot	Probable error expected with random selection of ultimate plots	Approximate size of plot (acre)	Shape of plot			Probable error of single plot	Probable error expected with random selection of ultimate plots
	Length	Width					Length	Width			
	Rods	*Rows*	*Feet*	*Per cent*			*Rods*	*Rows*	*Feet*	*Per cent*	
1/800	1	1	3⅓	10.607	10.607	1/400	2	1	3⅓	8.837	7.501
1/400	2	1	3⅓	8.837	7.501	1/200	2	2	6⅔	7.277	5.304
3/800	3	1	3⅓	8.083	6.124	1/100	2	4	13⅓	6.460	3.751
1/200	4	1	3⅓	7.686	5.304	1/50	2	8	26⅔	5.806	2.652
1/160	5	1	3⅓	7.087	4.744	1/25	2	16	53⅓	5.164	1.875
3/400	6	1	3⅓	7.053	4.331	2/25	2	32	106⅔	4.187	1.326
1/80	10	1	3⅓	5.921	3.355						
1/40	20	1	3⅓	5.100	2.372	1/200	4	1	3⅓	7.686	5.304
1/20	20	2	6⅔	4.312	1.677	1/100	4	2	6⅔	6.509	3.751
3/40	20	3	10	3.940	1.369	1/50	4	4	13⅓	5.812	2.652
1/10	20	4	13⅓	3.914	1.186	1/25	4	8	26⅔	5.314	1.875
1/5	20	8	26⅔	3.462	.839	2/25	4	16	53⅓	4.816	1.326
2/5	20	16	53⅓	3.024	.593	4/25	4	32	106⅔	3.865	.938
1/800	1	1	3⅓	10.607	10.607	1/160	5	1	3⅓	7.087	4.744
1/400	1	2	6⅔	8.479	7.501	1/80	5	2	6⅔	5.915	3.355
3/800	1	3	10	7.731	6.124	1/40	5	4	13⅓	5.244	2.372
1/200	1	4	13⅓	7.248	5.304	1/20	5	8	26⅔	4.793	1.677
1/160	1	5	16⅔	7.116	4.744	1/10	5	16	53⅓	4.280	1.186
3/400	1	6	20	6.720	4.331	1/5	5	32	106⅔	3.337	.839
1/100	1	8	26⅔	6.427	3.751	2/5	10	32	106⅔	2.373	.593
1/80	1	10	33⅓	6.378	3.355						
1/50	1	16	53⅓	5.552	2.652	1/80	10	1	3⅓	5.921	3.355
1/25	1	32	106⅔	4.458	1.875	1/40	10	2	6⅔	4.913	2.372
2/25	2	32	106⅔	4.187	1.326	1/20	10	4	13⅓	4.338	1.677
4/25	4	32	106⅔	3.865	.938	1/10	10	8	26⅔	4.094	1.186
1/5	5	32	106⅔	3.337	.839	1/5	10	16	53⅓	3.524	.839
2/5	10	32	106⅔	2.373	.593	2/5	10	32.	106⅔	2.373	.593

In a comparatively uniform field large plots are likely to be more reliable than small ones, notwithstanding that the advantage generally may be less than that indicated by the results of the usual blank experiments. Table 3 shows a comparison of probable errors when they are derived from a constant number of plots of increasing size and from all plots of the field of corresponding sizes. The error of a 1/800-acre plot when the first 48 ultimate plots are considered is 9.771 per cent, while it is 10.607 per cent when the entire block is used. The errors decrease in about the same proportion until a 1/20-acre plot is reached, when they are the same since the whole block is used in each case. In this instance, increasing the plot from 1/800 acre to 1/20 acre with the total area concerned the same reduces the probable error about 60 per cent, whereas a like increase in the size of the plot and a proportionate increase in its area reduces it 56 per cent.

On the assumption that the probable error derived from the first 48 ultimate plots might be excessively high, it was calculated for a like number at each ¼, ½, and ¾ of the distance across the block and also starting from the other side. The probable errors are shown at the bottom of Table 3. In two cases they are approximately the same as for the first trial; in one the error is actually a little higher than that found from all plots in the field, and in the other it is considerably lower.

The deviation of the actual curve of reduction in error from the expected (fig. 4) is similar to that usually found in blank experiments and has been explained in several papers.

TABLE 3.—*Reduction in probable error due to increase in size of plot with the number of plots constant compared with reduction when using the entire block*

Size of plot (acre)	Number of plots constant			Using entire block		
	Number of plots		Probable error (per cent of mean)	Number of plots		Probable error (per cent of mean)
	Ultimate	Combination		Combination	Ultimate	
1/800	48	(48)	[a] 9.771	1,920	1,920	10.607
1/400	96	48	8.172	960	1,920	8.837
1/200	192	48	6.678	480	1,920	7.686
1/80	480	48	5.305	192	1,920	5.921
1/40	960	48	5.051	96	1.920	5.100
1/20	1,920	48	4.312	48	1,920	4.312
1/800	48	(48)	[b] 10.656 [c] 9.795 [d] 7.217 [e] 9.881	1,920	1,920	10.607

[a] Starting with 3d row of Table 1.
[b] Starting with 26th row of Table 1.
[c] Starting with 51st row of Table 1.
[d] Starting with 74th row of Table 1.
[e] Last 48 rod rows ending with row 98.

SHAPE OF PLOTS

Plots of the same size but of different shape are compared in Table 4. There is no decided or consistent advantage of one shape over another.

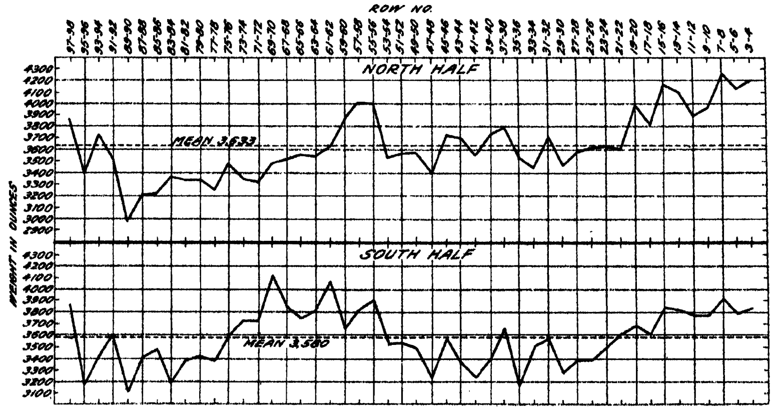

FIG. 5.—Variation in yield of 1/40-acre plots (10 rods by 2 rows) from the east side of the block to the west

This result may be accounted for in an examination of Figures 5, 6, and 7. Figure 5 is a graphic representation of yields of 1/40-acre plots (10 rods by 2 rows) from the east side of the field to the west; fluctuations in each are shown for the north half and the south half of the block. Figure 6 gives the variation from north to south; the block is divided into an east, middle, and west third, and the variation is shown for each section; the plots are 1 rod by 32 rows, or

1/25 acre. Figure 7 shows a division of the block into high, medium, and low areas based on the yields of 1/200-acre plots (1 rod by 4 rows).

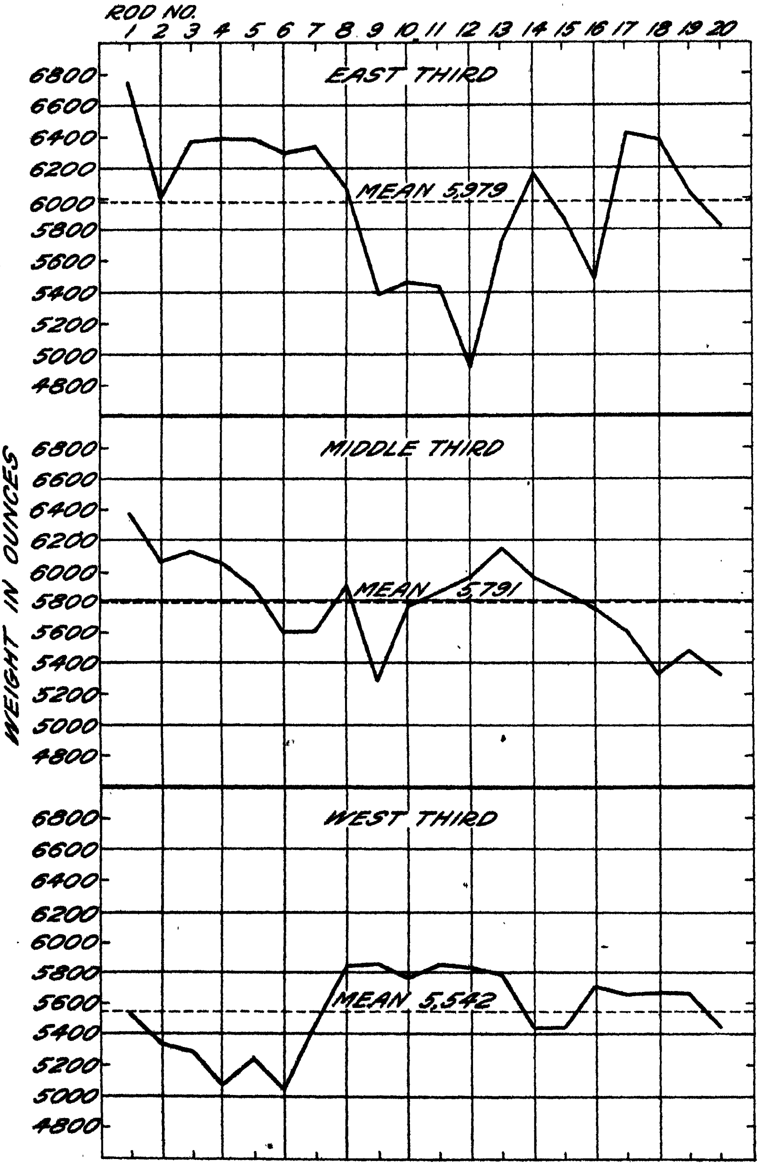

FIG. 6.—Variation in yield of 1/25-acre plots (1 rod by 32 rows) from the north side of the block to the south

This grouping is arbitrary, but it conforms rather closely with Figures 5 and 6 and may be taken as roughly correct.

There is some general decrease in yield from north to south and from east to west. But it is not markedly more variable in one direction than in the other, and so it would not be expected that there could be much difference in the probable error, regardless of the direction in which the length of plots was extended.

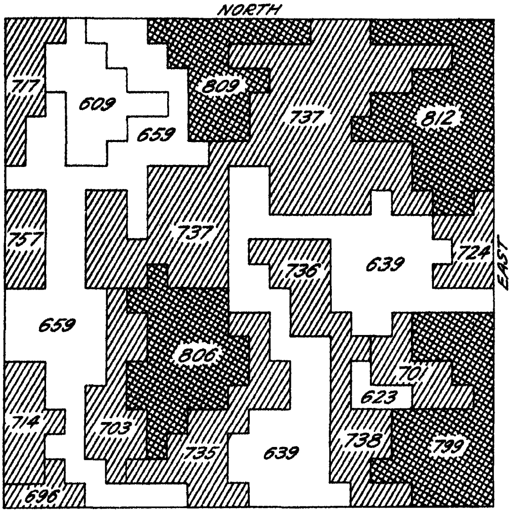

FIG. 7.—High, medium, and low yielding areas based on average yields of 1/200-acre plots (1 rod by 4 rows)

The outlines and the differences in means of the various groups in Figure 7 show that variation in this field was characteristically "spotted" rather than progressive from one side of the field to the other.

TABLE 4.—*Probable errors compared when plots are alike in size but different in shape*

Approximate size of plot (acre)	Shape of plot			Probable error of single plot (per cent mean)
	Length	Width		
	Rods	*Rows*	*Feet*	
1/400	2	1	3⅓	8.837
	1	2	6⅔	8.479
3/800	3	1	3⅓	8.083
	1	3	10	7.731
1/200	4	1	3⅓	7.686
	2	2	6⅔	7.277
	1	4	13⅓	7.248
1/160	5	1	3⅓	7.087
	1	5	16⅔	7.116
3/400	6	1	3⅓	7.053
	1	6	20	6.720
1/80	10	1	3⅓	5.921
	5	2	6⅔	5.915
	1	10	33⅓	6.378
1/50	4	4	13⅓	5.812
	2	8	26⅔	5.806
	1	16	53⅓	5.552
1/40	20	1	3⅓	5.100
	10	2	6⅔	4.913
	5	4	13⅓	5.244
1/25	4	8	26⅔	5.314
	2	16	53⅓	5.164
	1	32	106⅔	4.458
1/20	20	2	6⅔	4.312
	10	4	13⅓	4.338
	5	8	26⅔	4.793
1/10	20	4	13⅓	3.914
	10	8	26⅔	4.094
	5	16	53⅓	4.280
1/5	20	8	26⅔	3.462
	10	16	53⅓	3.524
	5	32	106⅔	3.337
2/5	20	16	53⅓	3.024
	10	32	106⅔	2.373

REPLICATION

Replication has been recommended and urged until rather few agronomy experiments involving but single plots are now in progress. The single-plot experiments of to-day are largely long-time tests that were established before so much emphasis was placed on replication.

The value and some of the difficulties of distributing plots have been considered in numerous papers, and part of the evidence has been derived from blank experiments. Generally a systematic method of distribution is used, because of the inconvenience in planting and harvesting with a random distribution. While not without exception, the curve of reduction in error by increased systematic replication usually approximates the curve expected from an increase in the number of plots with random distribution. If it does not, the system groups either similar or dissimilar yielding plots more often than would be expected from a random scattering. With the comparatively small numbers involved in field tests, even random distributions would result at times in deviations of considerable extent from those theoretically expected. However, the actual random distributions which have been reported have approached the expected more closely than the usual systematic replication.

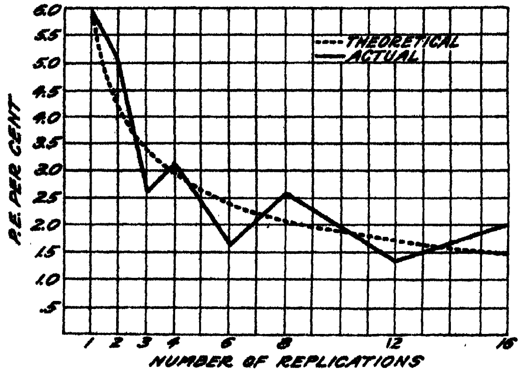

FIG. 8.—Reduction in probable error due to increased number of 1/80-acre plots systematically distributed and expected reduction due to increased number of plots distributed at random

Table 5 gives the probable errors of 1/80-acre and 1/40-acre single plots and of several replications[6] of each size up to 16; the expected errors with random distribution are added. Figures 8 and 9 show the same results. With each size the plots are 10 rods long, and since the block is 20 rods long there are two series across the field. The system of distribution made the units of a group in the second series fall halfway between the units in the first series.

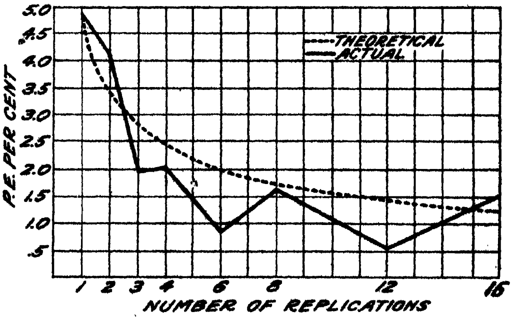

FIG. 9.—Reduction in probable error due to increased number of 1/40-acre plots systematically distributed and expected reduction due to increased number of plots distributed at random

Table 5 shows that the probable error has been reduced by replication. The reduction is not regular, however, and deviates at times rather widely from the theoretical. While sometimes above, the deviations are more often below the expected. This means that plots yielding dissimilarly were grouped by the system of replication more often than would be expected from random distribution. Such results indicate that the probable errors of systematically distributed plots often may be too low. There is no justification for concluding that three replications are more reliable than four simply because in this particular trial a lower probable error was obtained with three than with four. The system of replication used is one of many that could have been tried, and each would have given somewhat different results. The general tendency is for lower error with increased replication.

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[6] Number of replications is here used as the number of distributed plots and not the number plus 1.

TABLE 5.—*Reduction in probable error due to replication*

Number of replications	Probable error (percentage of mean) in—				
	1/80-acre plots (10 rods by 1 row)		1/40-acre plots (10 rods by 2 rows)		
				With systematic distribution	
	Expected with random distribution	With systematic distribution	Expected with random distribution	All of 96 plots	Number of groups constant at 16
Single	....	5.921	....	4.913	4.619
2	4.187	5.042	3.475	4.175	2.388
3	3.419	2.605	2.837	1.959	1.532
4	2.961	3.145	2.457	2.017	1.134
6	2.418	1.627	2.006	.844	.844
8	2.094	2.596	1.737	1.649	....
12	1.709	1.319	1.418	.540	....
16	1.480	2.022	1.228	1.522	....

The same criticism that has been made with respect to the evidence obtained from blank experiments favoring increased size of plot (see p. 638) may be made of this evidence in favor of plot replication, namely, that in practice the area of ground used must increase in proportion to the number of replications, and thus additional soil variations are included. The last column of Table 5 shows the probable errors of 1/40-acre plots with the number of groups constant at 16 and the area of land increasing in proportion to the number of replications. The reduction in this case is almost as great as when the errors are calculated from the entire block. Other trials starting from various points in the field would give somewhat different results, but probably they would show reduction even though less than would be found when using the entire block.

VALUE OF REPLICATION IN UTILIZING A GIVEN AREA FOR EACH VARIETY

In the previous tables the number of varieties or the area of land has changed with plots of different sizes or with numbers of replicated plots. If only a limited acreage is available and a specific number of units must be tested, it is desirable to know the best utilization which can be made of this area. Assuming 1/40 acre devoted to each unit of a test, Table 6 gives probable errors of single 1/40-acre plots and smaller systematically replicated plots which total

1/10 acre. The smaller plots were distributed in the same sequence in each replication. The 1/20-acre and 1/40-acre plots were the full length of the block, but the 1/80-acre plots were only 10 rods long, which allowed two series across the block, and the system of distribution made the units of a group in the second series fall halfway between the units in the first series. The 1/160-acre plots were 5 rods long, which allowed four series across the block. The distribution was arranged so that the units of a group in the second, third, and fourth series fell one-half, one-fourth, and three-fourths, respectively, of the distance between the units of a group in the first series.

The probable error is very little lower with 16 replications of 1/160-acre plots than with 4 replications of 1/40-acre plots. The reduction in the probable error of single 1/10-acre plots amounts to 33 per cent for 16 replications of 1/160-acre plots and 31 per cent for 4 replications of 1/40-acre plots.

Table 6.—*Reduction in probable error due to replication within a given area*

Number of replications	Area of single plot (acre)	Shape		Probable error (per cent of mean)
		Length	Width	
		Rods	*Rows*	
Single	1/10	20	4	3.914
2	1/20	20	2	3.725
4	1/40	20	1	2.710
8	1/80	10	1	2.596
16	1/160	5	1	2.626

DISCUSSION

Very often the uniform land provided for experimental purposes near colleges and universities is rather limited in proportion to the number of men who desire to conduct independent investigations. Under such conditions it may be desirable to reduce the area of land devoted to any one test as much as possible and refine the methods.

On branch stations and substations the situation frequently is different. There may be plenty of comparatively uniform land, since the location is not influenced by convenience for a college staff, but often there is only one trained man to direct all of the work. It is to his advantage to conduct the field tests in such a manner as will require the minimum of supervision commensurate with a reasonable degree of accuracy.

One means of reducing technical labor and supervision is to arrange the routine tests so that they may be handled with ordinary farm machinery with which the more or less transient laborers are familiar. It then becomes desirable to use reasonably large plots rather than small plots with a large number of replications, if the error can be kept within limits that will give significance to varietal and cultural differences.

An additional advantage of conducting tests with standard farm equipment is that there is less likelihood of error in the application of results.

General observations in an experiment of this nature are fraught with some danger. The results, however, should give an indication of the limits within which the size of plot and the number of replications should be confined. It is not to be assumed that probable errors for plots of the same size would be of like values with another crop, with the same crop in another year, or with the same crop on another block at the same station. Even a different system of replication with the same data would give somewhat different errors. Since the curve of actual reduction of error with increased replication is more often below the curve of expected reduction than above it, it would seem well to keep in mind the expected error.

In Table 2 the percentage of probable error of a single 1/10-acre plot is 3.914. Three times the error of a difference between two such plots is 16.6 per cent. Considering the error of three replications of 1/40-acre plots as 2.837 per cent, the expected error (three times the error of a difference between two replicated series) is 12 per cent, instead of 1.959 which was actually found (Table 5). Likewise for four replications of 1/40-acre plots and six replications of 1/80-acre plots, it is approximately 10 per cent. Additional replications necessary to reduce a significant difference below 10 or 12 per cent would hardly seem justified in sorghum-variety tests. Similar-yielding varieties often have other characteristics, such as resistance to lodging, fineness of stem, yield of grain in forage sorghums and forage in grain sorghums, and many other differences that influence the value of a variety even more than would a 10 per cent difference in yield.

Furthermore, it is usually assumed that a test should be continued for several years to include a sample of seasons. The magnitude of error from seasonal variation has been given little attention, except in a general way, but Engledow and Yule[7] and Stadler[8] have shown that it is rather large. They also have pointed out the futility of elaborate systems intended to reduce plot error to the minimum when seasonal error is not even measured.

SUMMARY

Green weights at maturity of the crop were taken on 2,000 rod rows of a selected uniform strain of feterita. The distribution of weights of 1,920 of these rod-row plots (borders excluded) formed a frequency polygon of the approximate shape of a normal curve. Plot variation as measured by the probable error of a single plot in percentage of the mean was determined for the rod-row plots and for larger plots consisting of various combinations of rod rows.

The probable error of a single rod row or 1/800-acre plot was 10.607 per cent, but this error was reduced consistently by taking successively larger plots of the same general shape. The error of a 2/5-acre plot was 3.204 per cent.

Owing to some decrease in yield both from north to south and from east to west and to the spotted tendency of high-yielding and low-yielding areas, no particular advantage of one shape of plot over another was found.

Systematic replication was effective in reducing error, but the reduction was irregular. Very often the error found was considerably below that which would be expected from a random distribution of the same number of plots.

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[7] Engledow, F. L., and Yule, G. U. THE PRINCIPLES AND PRACTICE OF YIELD TRIALS. Empire Cotton Growing Rev. 3: 112-146, 235-267, illus. 1926.

[8] Stadler, L. J. Op. cit.

In the trials made, the error was reduced nearly as much when the area of land was increased in proportion to the size of the unit plot or in proportion to the number of replications as it was when determined from the whole block.

Results of the test indicated that three or four replications of $\frac{1}{40}$-acre or $\frac{1}{80}$-acre plots will give results sufficiently reliable for the ordinary sorghum test.

SOME CHEMICAL AND MORPHOLOGICAL PHENOMENA ATTENDING INFECTION OF THE WHEAT PLANT BY OPHIOBOLUS GRAMINIS[1]

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INTRODUCTION

Take-all of wheat, caused by *Ophiobolus graminis* Sacc., was first discovered in the United States in Virginia in 1919. Since that time it has been found in most of the winter-wheat areas of this country. During the last few years some work has been, done on the disease and the causal fungus by Davis (*2*),[2] Kirby (*3*), McKinney and Davis (*4*), Webb and Fellows (*11*), and others in the United States. In Australia considerable attention has been given to take-all, and in France some work has been done on what is thought to be the same disease. However, Davis (*2*) is the only worker who has studied the microscopical and macroscopical phases of infection. The nature of Davis's work is briefly described in his summary as follows:

> Histological studies were made from plants exhibiting various stages of infection and these showed that the parasite entered the unbroken epidermis of the underground portions of the leaf sheaths, culms, and roots. The parasite first destroys the cortex of the roots and later enters the central cylinder. It also destroys the leaf-sheath and culm tissues and later enters the vessels, but it does not appear to make much progress after it enters the vessels.

The work on infection described in the present paper has been of two kinds, (1) morphological and histological and (2) microchemical. The purpose of the histological study was to learn what tissues were invaded and what morphological changes occurred in these tissues. The composition of the cell walls before and after infection was determined by microchemical methods. The roots, subcoronal internode, and crown were used in the histological study, but only the roots were employed in the microchemical studies. The results of the work described in this paper already have been a valuable aid in interpreting the behavior of wheat plants affected with take-all.

MATERIALS AND METHODS

Most of the histological studies were made with material embedded in paraffin. A combination of safranin and light green was found to be the best stain for differentiating the host and the parasite. This method of staining was especially satisfactory in lignified tissues where the host cell walls stained red and the fungus green.

Phloroglucin, used according to the method given below, was the best reagent for the detection of the fungus in the cell wall itself. Thin sections of fresh tissue were first treated 10 minutes in hot

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[1] Received for publication Aug. 24, 1928; issued December, 1928. These investigations were conducted in cooperation with the Kansas Agricultural Experiment Station, Manhattan, Kans. Paper No. 258 of the Department of Botany and Plant Pathology.

[2] Reference is made by number (italic) to "Literature cited," p. 661.

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hydrogen peroxide with frequent changes. The sections were then transferred to a 1 per cent solution of alcoholic phloroglucin on a glass slide and allowed to remain until the solution had mostly evaporated. One or two drops of concentrated hydrochloric acid then were applied, the cover slip placed in position, and the sections observed. Lignified cell walls showed red after this treatment, whereas the fungus hyphae passing through the walls showed as dark lines.

Kanred wheat was used in all of the studies described in this paper. Where constant temperatures were maintained, soil-temperature control tanks, of the type developed in Wisconsin, were used.

Microchemical tests were made according to mimeographed directions received from Sophia H. Eckerson of the Boyce Thompson Institute for Plant Research (Inc.), Yonkers, N. Y. Other supplementary references were used (*1*, *9*). Briefly, some of the principal microchemical tests employed were as follows:

CELLULOSE: (1) Stain.—Iodine-potassium iodide + 75 per cent sulphuric acid. (2) Solubility.—Copper oxide ammonia, 50 per cent chromic acid, zinc chloride and hydrochloric acid about 1:2.

HEMICELLULOSE: (1) Stain.—Furfural reaction with 1 per cent phloroglucin in alcohol and concentrated hydrochloric acid + heat; 4 per cent orcin and concentrated hydrochloric acid + heat. (2) Solubility.—Hot 3 per cent sulphuric acid.

CALLOSE: (1) Stain.—Resorcin blue (1:1,000), aniline blue dilute solution. (2) Solubility.—Cold 1 per cent sodium hydroxide or potassium hydroxide.

PECTIC SUBSTANCES: (1) Stain.—Ruthenium red (1:10,000 in water); methylene blue (1:1,000 in water). (2) Solubility.—Two per cent hydrochloric acid changes pectose to pectin or pectic acid; 2 per cent ammonia dissolves pectic acid.

LIGNIN: (1) Stain.—One per cent phloroglucin in alcohol + 25 per cent hydrochloric acid; phenol-potassium chlorate mixture followed by concentrated hydrochloric acid. (2) Solubility.—Fifty per cent chromic acid. (3) Oxidation.—Hydrogen peroxide, potassium chlorate.

FURFURAL: (1) Stain.—Furfural reaction. (2) Removal.—Hydrocyanic acid.

SUBERIN: (1) Stain.—Sudan III. (2) Solubility.—Three per cent alcoholic potassium hydroxide. Insoluble in 50 per cent chromic acid.

MORPHOLOGICAL AND HISTOLOGICAL STUDIES

PRIMARY ROOTS

DESCRIPTION

The primary roots originate from the hypocotyl of the embryo and are the roots upon which the young plant depends for the intake of water and mineral nutrients before the crown and secondary or permanent roots arising from it are formed. The primary roots do not necessarily end their usefulness to the plant when the permanent roots are formed. Their duration as useful organs after this time varies according to conditions. Sometimes the primary roots may still be functioning when the plant is mature. On the other hand, many of the primary roots may die and disintegrate soon after the secondary roots are formed.

A cross section of a young primary root shows it to be composed generally of three parts—epidermis, cortex, and stele. (Fig. 1.) The epidermis consists of a single layer of elongated thin-walled cells. It is from these epidermal cells that the root hairs are developed and it is through the epidermal cells that infection occurs. The cortex lies just beneath the epidermis and is composed of four or five layers of large, thin-walled cells. The endodermis, the innermost layer of the cortex, consists of a single, continuous layer of closely fitting cells. The outer tangential walls of the endodermal cells are thin, while the radial and inner tangential walls are thickened.

The pericycle, the outermost layer of the stele, consists of a single layer of radially elongated cells. The walls of the pericycle cells are slightly thickened. All the cells are nearly equal in size, except those opposite the xylem, which are smaller.

The xylem strands are seven or eight in number and alternate with the phloem. The protoxylem vessels are strengthened with spiral thickenings, but those formed later toward the center are pitted. The phloem bundles consist of series of three thin-walled cells. The conjunctive tissue of the stele consists of thin-walled irregular parenchyma cells. In the center of the stele is found a large central vessel with a heavy wall.

INFECTION

It is well to mention here that the hyphae of *Ophiobolus graminis* are of two kinds, distinguished by color and size. The macrohyphae are large in diameter, thick walled, and dark in color. The microhyphae are more slender, thinner walled, and colorless. The macrohyphae are ordinarily, though not always, found on the outer surface of tissues, and the microhyphae are generally found within the cells.

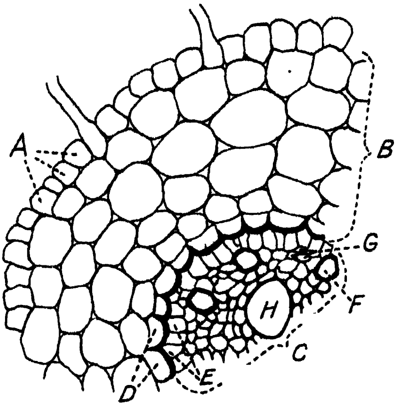


FIG. 1.—Sector of a cross section of a healthy primary root of a wheat plant. A, Epidermis; B, cortex; C, stele; D, endodermis; E, pericycle; F, xylem; G, phloem consisting of groups of three cells; H, central cavity. × 231

The macrohyphae, as they are ordinarily found on the roots, grow in contact with the epidermal cells. Where the macrohyphae are thus in contact with the epidermal cells, microhyphae may branch off at any point and penetrate the epidermal cells of the roots. The original penetration from the outside may or may not be attended with constrictions of the hyphae at the point of entrance. Once inside the root, the hyphae seldom undergo a change in size when passing through a cell wall except in instances where the cell wall undergoes a change in the immediate presence of such hyphae. These changes will be described later in this paper.

After entering the cortex the hyphae grow intracellularly from cell to cell in a radial or obliquely radial direction. In the cells of the cortex they have never been observed to parallel the long axis of the root. While the endodermis offers some resistance to the radial growth of the fungus, various segments of it differ in this respect. Certain portions may offer no resistance and others much. Finally the hyphae enter the stele and may penetrate any of its cells. A small lesion on one side of a root is sufficient to permit all of the vital conducting regions of the stele to become infected. The fungus that has entered one side of a root and has penetrated into the stele has never been observed to pass out of the stele into the cortex on the side opposite the primary infection.

After entry into the stele the hyphae show a tendency to grow lengthwise of the root. This occurs especially in the xylem tubes or in the spaces created by the disintegration of the parenchyma and phloem of the stele.

It is well to mention here that a short time after complete or incomplete occupancy of the root the hyphae die and disintegrate completely. Therefore, the only indications of the earlier presence of the fungus are the malformations on the remaining cell walls or the destruction of cell contents and cell walls. The stele may or may not have been entered before this disintegration occurred.

The foregoing description may give the impression that the advance of the fungus through the invaded tissue is always fairly regular, but such is not invariably the case. In some cells the hyphae become aggregated much more than in others. The wall of a cell thus filled with mycelium becomes greatly thickened, making it difficult for the hyphae to pass from it to the next cell. In other cases the progress of a single hypha is in nearly a straight line so that it may be traced through four to five cells in succession. These variations in the behavior of the fungus may be due either to differences in food supply in the different cells or to mechanical or other differences in the nature of the cell walls themselves. In the former case the hyphae may remain and branch abundantly in the cells where the food is abundant, while in the latter case the hyphae may find it difficult to escape from some cells and thus be forced to remain. Aggregations of hyphae occur not only in single cells but also in groups of adjacent cells. Judging from the variations in behavior of the fungus in different cells, it seems that cells of the same tissue differ individually.

SECONDARY ROOTS

DESCRIPTION

The secondary roots, which form the bulk of the root system, are formed at or near the crown of the plant. Their number is not limited to a few (usually three), as is true of the primary roots. The crown is located at or somewhat below the surface of the ground and is the region in which several nodes are closely crowded together and from which tillers and leaves as well as secondary roots are formed. Secondary roots are formed not only in the original culm of the plant, but also grow from basal nodes of the tiller culms. New secondary roots may be forming for a considerable time during the early growth of the wheat plant.

The anatomy of the secondary roots does not differ greatly from that of the primary, except that their diameters are greater because of the greater quantity of parenchymatous tissue in the cortex and more abundant conjunctive tissue in the stele. As a secondary root becomes older, the walls of the outermost two or three layers of cortical cells become thickened. This thickening occurs first at the base of the root, that is, in the older portion, and gradually progresses toward the tip.

INFECTION

Infection and progressive penetration of the secondary roots by the fungus do not differ materially from those of the primary roots, with one exception. Within the thick-walled cortical cells, mentioned above, and in some of the cortical cells immediately outside of these, the hyphae become aggregated in large numbers, assume the form of macrohyphae, and grow lengthwise in the cells. These macrohyphae may become so numerous in a cell as to cause it to bulge and break. Furthermore, by means of microhyphae, the macrohyphae may penetrate outward through the cell wall, in which they are inclosed, in any direction rather than only toward the center of the root.

COLEOPTILE

DESCRIPTION

The coleoptile forms a complete sheath for the young growing plumule, its only opening being a slit near the apex. It is in reality a leaf folded longitudinally on itself with the two margins united. Within the cavity of the coleoptile is located the plumule.

In cross section the coleoptile is oval. At each extremity in the oval is a vascular bundle. The outer epidermis is composed of small cells more or less radially elongated and with their outer tangential walls slightly thickened. The cells of the inner epidermis, which are in more or less direct contact with the plumule, are similar to the cells of the outer epidermis except that they are elongated tangentially and the walls are not thickened. The tissue between the outer and inner epidermis is composed of large parenchyma cells with the exception of the two vascular bundles mentioned above. These parenchyma cells will be called in the present paper mesophyll. (Pl. 1, B.)

INFECTION

As the coleoptile becomes infected, macrohyphae are found closely appressed to the outer epidermis with their long axes parallel to the long axis of the coleoptile. These hyphae may be piled on one another many layers deep. Now and then a macrohypha ends off a side branch toward the epidermis. At the junction of the attacking hyphae and the attacked cell the hyphae may still be macrohyphae. As penetration of the host cell wall is effected a marked constriction occurs in the penetrating hypha. After emergence on the inner side of the wall the hypha may or may not enlarge somewhat.

Penetration continues through the mesophyll to the inner epidermis. When the inner epidermis of the coleoptile is reached by the hyphae they accumulate in great abundance in that vicinity and apparently are unable to proceed farther. Sometimes the inner epidermis may be infected, but more often it is not. The writer has never seen a case where the hyphae have penetrated the young leaves from the coleoptile, nor observed any lesions on the leaves as they emerge from the coleoptile. Seemingly the coleoptile acts as a protective organ for the inclosed young plumule during its early stages. A similar kind of protection has been noted by Reed and

Melchers (7) with respect to the attack of milo and feterita seedlings by *Sphacelotheca sorghi*.

It is true that the subcoronal internode, which often is infected, is surrounded by the coleoptile. However, the coleoptile has become ruptured by growth processes and often is mostly disintegrated and therefore can offer no further protection.

SUBCORONAL INTERNODE

DESCRIPTION

The subcoronal internode is the underground part of the stem of the wheat plant, below the crown. It is called a rhizome by Percival (5). On its upper end is the crown, and at its lower extremity are the primary roots and the remains of the seed.

A view in cross section shows the subcoronal internode to consist of essentially four parts, (1) epidermis, (2) cortex, (3) a cylinder of vascular bundles, and (4) the pith in the center. (Fig. 2.) The epidermal cells are closely appressed to one another and are consequently more or less square in cross section or elongated radially. The outer and radial walls are heavily lignified. The cortex has a thickness of six or seven layers of cells, the outer cells small, but gradually increasing in size toward the center. These cells are not especially thick walled. Occasionally there are groups of sclerenchyma cells in the cortex.

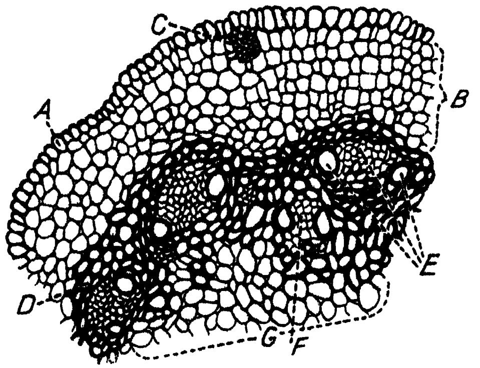


FIG. 2.—Portion of a cross section of the subcoronal internode of a healthy wheat plant. A, Epidermis; B, cortex; C, group of sclerenchyma cells; D, groups of thick-walled cells connecting with one another and making a complete cylinder surrounding the bundles and pith; E, large bundle; F, small bundle; G, pith. × 96

The bundles are arranged in a circle of seven large ones alternating somewhat irregularly with seven smaller ones. Each bundle is surrounded by thick-walled lignified cells, which are joined from bundle to bundle, thus forming a continuous irregular circle inclosing all the bundles and the pith. The pith is composed of large, thin-walled parenchyma cells loosely joined together.

INFECTION

Infection of the subcoronal internode is similar to that of the roots; that is, infection occurs through the epidermis, at any point. The hyphae proceed though the cortex and bundle sheaths into the pith. Any cell may become infected. The hyphae, after entering the xylem tubes and thick-walled cells of the bundle sheaths, assume the form of macrohyphae and grow lengthwise in these cells. This lengthwise growth of a single hypha has been traced as far as 1 cm. and doubtless may extend farther.

CROWN

The primary purpose of the present study of the crown was to learn the behavior of the crown and of the fungus in the crown of plants that were able to recover from the take-all disease. The crown is of particular interest from this standpoint because in such plants it has been only partially invaded. In a recovered plant the primary roots, the subcoronal internode, the first, second, and third leaves, and some of the secondary roots, expecially the lower ones, all may be destroyed, while the upper part of the crown and the higher secondary roots may be free from the disease. Such recovered plants are able to grow to maturity and produce a normal head. As pointed out by Davis (*2*), such upper secondary roots no doubt may escape infection on account of their later formation. The crowns of recovered plants are infected only in part. As soon as infection becomes general in the crown the entire plant dies and no recovery is possible.

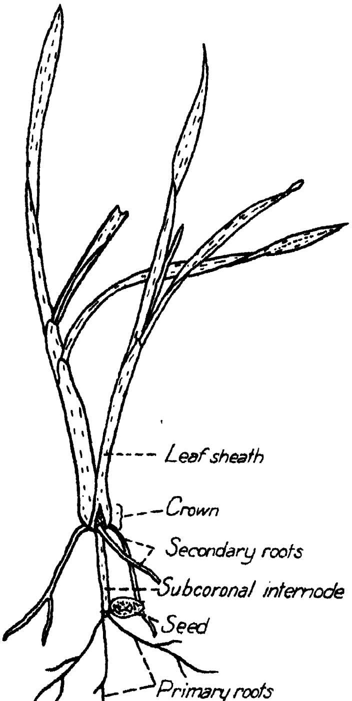


FIG. 3.—Diagram of a young wheat plant illustrating the location of the various parts. Subcoronal internodes may vary greatly in length

DESCRIPTION

The crown is the region in which the tillers, leaves, and secondary roots are formed. (Fig. 3.) The elongation of the culm, however, carries some of the leaves up away from the crown. The crown is surrounded by the sheath of the first leaf and partially also by the sheaths of the other lower leaves.

The lower true leaves and secondary roots, which latter usually come in pairs, alternate with each other vertically on the stem. The first true leaf comes at the junction of the subcoronal internode and the crown. The first two secondary roots come just above the attachment of the first leaf, one on each side at points about 90° from the middle of the base of the leaf. In growing outward each of these two secondary roots penetrates the base of the first leaf and thus ruptures it at two points. The second and other successive lower leaves alternate with the second and other pairs of secondary roots in a similar manner. Thus the leaves are two ranked on the stem, and the second and other successive pairs of secondary roots come approximately at right angles to each preceding pair.

The first, second, and third leaves receive their vascular traces directly from the bundles found in the subcoronal internode. The trace of the fourth leaf, however, does not connect directly with the vascular strands found in the subcoronal internode, but with branches of these. These branches come nearly at a right angle and meet the traces of the fourth leaf near the center of the crown. The fourth leaf, however, does not depend entirely upon the branches of the bundles from the internode for its supply of water and nutrients, for it is also connected with the vascular systems of the second pair of secondary roots. Subsequent leaves are connected with the vascular systems of the second and third pairs of secondary roots by means of branches of bundles sent from these roots to near the center of the crown where they connect with the leaf traces.

As pointed out above, the first two secondary roots come from the crown just above the attachment of the first true leaf. Their vascular systems connect directly with the established system coming from the subcoronal internode. The vascular systems of the second pair of secondary roots also connect directly with the bundles from the subcoronal internode and also establish an independent vascular system extending up the culm. This independent system branches toward the center of the crown, connecting with the fourth and fifth leaf traces and extending into the apical bud.

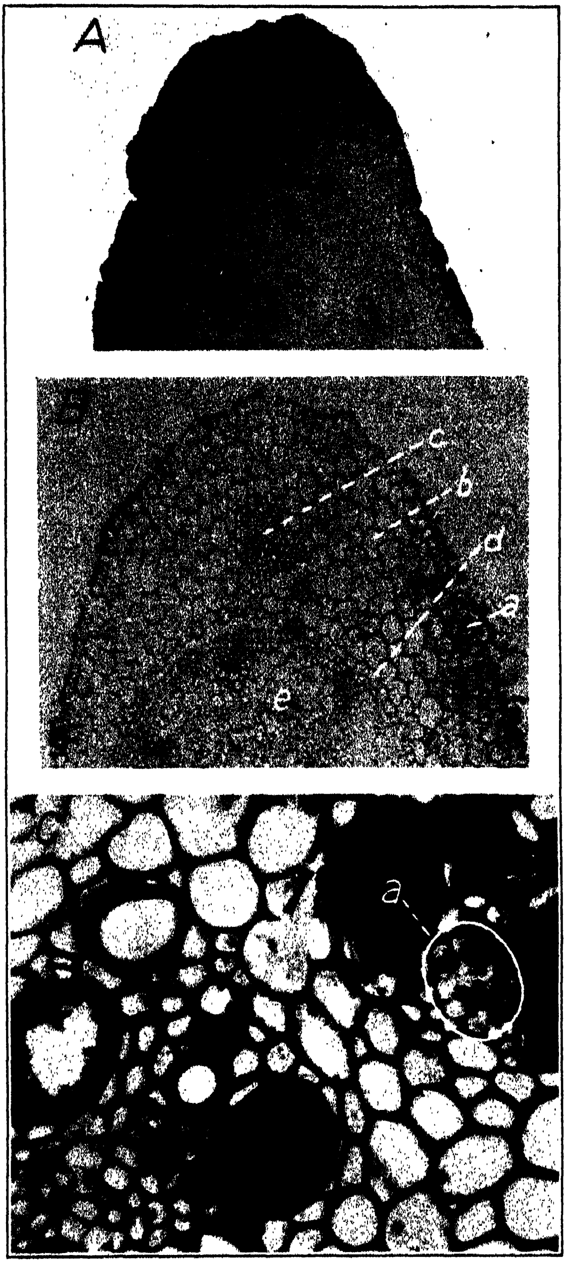
The vascular systems of the third pair of secondary roots have no connection with the bundles from the subcoronal internode but are independent and branch toward the center of the crown, connecting with leaf traces and extending to the apical region.

INFECTION

As noted above in discussing the infection of the subcoronal internode, the parenchyma cells of the cortex may become infected. There is an extension of these parenchyma cells into the crown for a short distance, but for some reason there is a sharp line of demarcation between the infected cells in the subcoronal internode and the uninfected cells in the crown. The parenchyma cells of the pith also extend into the crown. These cease in the region where the vessels from the subcoronal internode branch toward the center to connect with the traces of the fourth leaf. The latter parenchyma cells are infected as far as they extend into the crown but no farther.

If one cuts a longitudinal section in a median plane of the crown of a recovered plant, this pith region with its darkened infected cells may be seen to extend into the crown in a cone-shaped area. The apex of the cone is just below the branches of the bundles from the subcoronal internode to the traces of the fourth leaf.

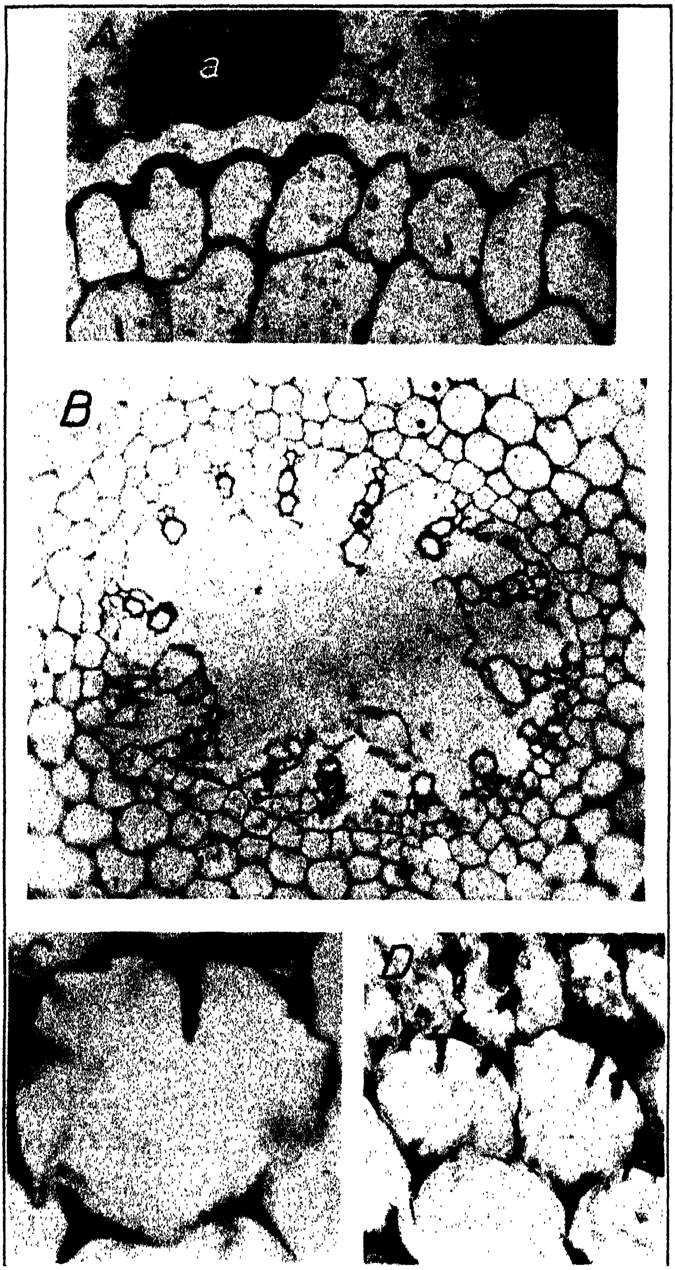
The hyphae in the cortical cells of the subcoronal internode extend out into the parenchyma cells of the first leaf. The hyphae growing lengthwise in the xylem cells of the subcoronal internode grow up into the xylem of the first leaf and to some extent may pass into the vascular strands of the first pair of secondary roots. It is noticeable that the hyphae become fewer and fewer in the xylem strands as the top of the subcoronal internode is approached. This diminution is more marked as they enter the crown. The same is true of the hyphae that enter the crown through the secondary roots. The reason for this will be discussed under a separate heading.



A.—Portion of a cross section of an infected coleoptile in which the mesophyll is disintegrated. The outer epidermis and most of the first layer of cells beneath it, the inner epidermis, the bundle, and the cells in its vicinity are not disintegrated. × 57

B.—Portion of a cross section of an infected coleoptile in which the cells of the mesophyll are not disintegrated: *a*, Outer epidermis; *b*, mesophyll; *c*, one of the two fibrovascular bundles; *d*, inner epidermis; *e*, leaves of the plumule. × 67

C.—Portion of infected vascular region of the subcoronal internode. The xylem tubes have become partly or completely clogged by products from their own walls; *a*, disintegrated phloem. × 500



A.—Portion of outer epidermis of the coleoptile showing thickening of outer tangential walls of epiderma cells caused by the incrusting layer of hyphae; the epidermal cells are not penetrated. The layer of hyphae *a* had been somewhat pulled away from the epidermis in the preparation of the section. × 330

B.—Stele of a primary root largely disintegrated by *Ophiobolus graminis*, with the xylem tubes and some of the parenchyma and pericycle cells in their immediate vicinity still intact; the walls of the endodermis have become thin. × 400

C and D.—Lignitubers in the cortical cells of roots of young wheat plants. × 1,000

MORPHOLOGICAL CHANGES IN CELL WALLS IN BASAL PORTIONS OF INFECTED PLANTS

CHANGES IN THE ROOTS

The morphological changes in the cell walls of the primary and secondary roots are very similar and will be discussed together. Changes that occur in the wall of one cell may be very different from those that occur in another, although the two cells may be comparatively close to each other, of a similar type, and have equal quantities of hyphae in them. Accordingly, the description of a change in a certain cell wall can not apply to the walls of all infected cells of that particular type.

Shortly after or even before the entry of mycelium into a cell its wall may become very much thickened. This thickening may or may not be uniform on all surfaces of the wall. It is especially noticeable at the corners. The thickening is not necessarily formed on the side of the wall toward the lumen, for it may form between the cells, or the intercellular spaces may be filled with it.

Somewhat similar cell-wall thickenings in diseased plant tissues have been observed by other investigators. Ravn (*6, p. 113*) described and figured cell-wall thickenings in oats attacked by *Helminthosporium avenae* Eidam. The composition of these thickenings was not definitely determined, although it was stated that they stained intensely with thionine and other basic stains.

Tisdale (*10*), working with flax wilt, pointed out that certain cell walls of the flax plant may become thickened in advance of the hyphae of *Fusarium lini* Bolley, and he attributed the thickening to the formation of suberin.

When a wheat root becomes infected with *Ophiobolus graminis* very noticeable changes occur in the walls of the infected cells in some cases. When an infecting hypha starts to penetrate a cell wall a slight protuberance is formed on the cell wall at a point just opposite. The protuberance elongates at right angles to the wall, becoming longer and longer in front of the hypha as the latter advances. (Pl. 2, C andD.) As the hypha progresses it becomes more and more attenuated. Finally either the hypha is able to outgrow the protuberance and enter the lumen of the cell or the protuberance is able to prevent its doing so. If the hypha passes through the protuberance, the hypha immediately increases in diameter to normal size again.

Protuberances in wheat cells caused by other organisms but similar to those described above have been observed by others, and the names callosities and calluses have been applied to them (*8, 12*). Either of these names would be a misnomer if applied to the abnormalities produced in wheat by *Ophiobolus graminis,* as microchemical tests have shown that the protuberances here described contain no trace of callose but rather are composed chiefly of lignin. Therefore the writer suggests the name "lignitubers" for the protuberances caused by *O. graminis*, the name alluding to their composition and form. This designation will be used in the present paper.

In form the large lignitubers have the general shape of a finger. (Fig. 4.) The surfaces are generally smooth and slightly undulating. The bases flare out and are joined to a similar substance on the inner surface of the cell walls. The same substance may occur also between the walls of the adjoining cells where the hypha penetrates. When the lignituber is viewed sideways a lighter staining median line may be seen extending lengthwise through or almost through it. This line is the hypha contained within. When viewed in cross section a lignituber resembles a doughnut, the central hypha corresponding to the hole in the doughnut. In size these lignitubers may vary greatly. Some are very minute; others are sufficiently long to cross completely the cell lumen.

Lignitubers do not invariably accompany penetrating hyphae. They are usually found most abundantly on walls of cells that thicken their walls otherwise in the presence of the attacking organism, and they may occur in cells of all the different tissues of the root.

In part of the cortex of the proximal portion of the secondary roots, about 0.5 cm. from the crown, it was found that the hyphae after entering certain cells often grew lengthwise rather than always penetrating the cells crosswise—that is, in the radial direction—as was the usual procedure in portions of the roots farther from the crown. In seeking an explanation for this behavior of the hyphae in those particular cortical cells close to the crown the corresponding tissues of a healthy root were examined. It was found that there occurred in the cortex of the root, near the crown, at a depth of one or two layers of cells below the epidermis, a ring of cortical tissue with rather thick cell walls. This ring was three or four cells in thickness. It seemed that the ability of these cortical cells to thicken their walls might account for the rather peculiar behavior of the hyphae in this tissue. This might occur in two ways. Hyphae that were in these cells when the thickening was taking place found difficulty in escaping radially and as a result grew lengthwise in the cells; or these cells, because of their inherent ability to thicken, were able to produce thick walls quickly, due to the stimulation of the fungi within, and thus forced the hyphae to grow lengthwise in the cells.

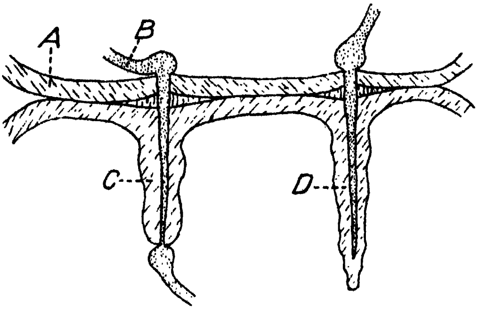


FIG. 4.—Diagram showing penetration of cell walls by infecting hyphae and lignitubers formed about them. A, Cell wall; B, hypha; C, lignituber through which the hypha has passed; D, lignituber through which the hypha has not passed. (See pl. 2, C and D.) × 3000

After the stele of either the primary or the secondary roots becomes invaded a disintegration of certain of the cells within may occur. When this happens the phloem and most of the parenchyma of the stele disintegrate, the phloem usually more rapidly than any of the other tissues. The walls of the xylem vessels, as well as the walls of the parenchyma and pericycle cells relatively close to the xylem, remain intact. (Pl. 2, B.) Such disintegration does not always occur, but it usually does. In cases of such disintegration the distal portion of the root beyond the lesion, of course, functions no longer.

Another notable change occurring in the bases of such infected secondary roots is the clogging of the xylem vessels and sometimes of the surrounding cells. The walls of the xylem vessels may become thickened to such an extent that the lumen often is entirely filled.

CHANGES IN THE COLEOPTILE

In a preceding paragraph it was mentioned that the macrohyphae accumulate in large numbers on the outer epidermis of the coleoptile. The presence of these hyphae, before they penetrate the cell walls of the epidermis, causes these walls to become greatly thickened with lignified material. (Pl. 2, A.) This thickening is most pronounced on the outer, tangential walls, but it also extends down the radial walls. When penetration of the epidermal wall occurs the hyphae apparently are always accompanied by lignitubers, many of which are larger than those seen in any other cells. The row of cortical cells immediately below the epidermis reacts similarly but not to such a marked degree. The walls of the other cells of the coleoptile mesophyll neither become thickened nor form lignitubers in the presence of the hyphae. The cell walls of the inner epidermis become slightly thickened but apparently do not form lignitubers.

Comparatively soon after infection the mesophyll cells of the coleoptile disintegrate completely with the exception of those near the two bundles. After this disintegration the two empty spaces thus formed appear as two crescents with their concave sides toward each other. The two bundles with the immediately surrounding mesophyll separate the two ends of these crescents. (Pl. 1, A.)

CHANGES IN THE SUBCORONAL INTERNODE

As stated previously, in a healthy plant the outer tangential walls of the epidermal cells are thickened. Apparently no additional thickening occurs after penetration by the fungus. On the other hand, the cell walls of any or all of the cells of the cortex may become thickened after penetration. This thickening is especially noticeable in the intercellular spaces. These become completely filled with a substance staining strongly with safranin.

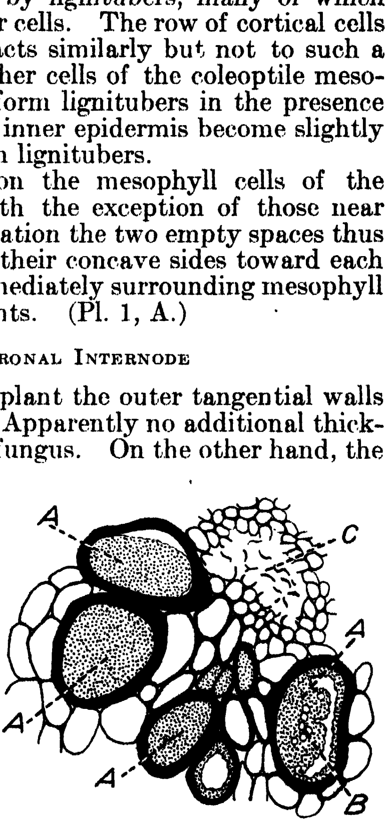


FIG. 5.—A diseased vascular bundle of the subcoronal internode. A, Xylem tubes that have become clogged or partly clogged with an outgrowth of their own walls, some of the smaller cells in the vicinity having also become clogged or partly clogged; B, a group of hyphae that are surrounded by the clogging material within a xylem tube; C, disintegrated cells of the phloem region. (See pl. 1, C.) × 400

The greatest changes that were noticed after infection were in the xylem tubes and some of the thick-walled cells surrounding the bundles. It was found that the walls of these cells became thickened to such an extent that the lumen became entirely filled. Most of the xylem tubes became completely plugged. This plugging occurred in advance of the hyphae or when they were present. (Pl. 1, C, and fig. 5.) In the latter case it was noticeable that the hyphae became disintegrated. Undoubtedly such plugging hinders considerably the upward progress of the hyphae into the crown. In this way the plant may be helped to recover from the disease, provided secondary roots are formed in large enough numbers to provide for the needs of the young plant after the primary roots have been cut off by the plugging of the vessels leading through the subcoronal internode.

As was the case in the roots, the phloem of the bundles in the subcoronal internode, as well as the pith, often disintegrates in the presence of the parasite.

CHANGES IN THE CROWN

As previously noted, studies of the crown have been made only on plants that have recovered from the disease. In such plants the hyphae have entered the crown through the xylem tubes of the subcoronal internode and lower secondary roots or through the pith of the subcoronal internode. The progress of the hyphae entering through the xylem tubes is so much hindered by the great thickening of the walls and subsequent plugging of these vessels that further progress in some tubes may be stopped. The hyphae entering through the pith disintegrate this tissue in the crown to a point just below where the vessels from the subcoronal internode branch off to the fourth leaf. The further progress of these hyphae is probably hindered by the rather thick, relatively resistant cell walls in the region of the branching.

MICROCHEMICAL STUDIES OF HEALTHY AND DISEASED ROOTS

As previously noted, infection of the basal portions of the wheat plant by *Ophiobolus graminis* is accompanied by certain morphological changes. Certain cell walls become thickened, and peculiar outgrowths called lignitubers are formed. It was the object of the microchemical studies, the results of which are here summarized, to determine what chemical changes, if any, occurred in the walls of the infected cells, (1) where morphological changes were evident and (2) where no morphological changes were noticeable.

The plants used in these studies were, on an average, 9 days old. They were grown in steamed soil subsequently infested with a pure culture of *Ophiobolus graminis* isolated from wheat in Kansas. The infested soil was placed in large test tubes, and disinfected seed of Kanred wheat was sown in it. These tubes were then kept at a temperature of about 20° C., and the moisture of the soil was kept at about 55 per cent of its moisture-holding capacity. The results are given in Table 1.

Table 1.—*Comparison of cell-wall constituents of healthy and diseased primary and secondary roots of young Kanred wheat plants grown in large test tubes at about 20° C.*

Cell-wall constituents in healthy roots	Cell-wall constituents in diseased roots
CELLULOSE	
All of the cell walls, including those of the root hairs, had their foundations of cellulose.	In general some cellulose was present in all cell walls, but it varied more in diseased than in healthy roots. Many cell walls of the cortex had lost all or part of their cellulose. The endodermis and parenchyma of the diseased stele had less cellulose than the healthy material. The lignitubers had no cellulose so far as tests showed. The pericycle of the diseased plants had little cellulose in comparison to that of healthy plants.
HEMICELLULOSE	
The only form of hemicellulose present was pentosans, found in the xylem.	No change was produced by disease.
CALLOSE	
The test for callose was negative.	None was found. Evidently the disease did not cause its production.
PECTIC SUBSTANCES	
Pectose was the only pectic substance present. It was located in the root hairs and epidermal cell walls.	No change was produced by the infecting organism.
LIGNIN	
Lignification occurred only in the walls of the xylem tubes.	The walls of the cortical cells that were thickened in the presence of the organism showed large amounts of lignin and comparatively little cellulose. The lignitubers and the substance filling the intercellular spaces in the cortex were largely lignin; likewise some striations in the inner tangential walls of the endodermis showed lignin. The parenchyma of the pith and the pericycle near the xylem had become lignified.
SUBERIN	
Suberin was present in the xylem and radial walls of the endodermis. In the xylem it was located in the thickenings.	A slight amount of suberin had been produced in the thickenings of the infected walls of the cortex. There was also a slight quantity in the lignitubers. Neither the cell wall thickenings nor the lignitubers were completely soluble in chromic acid, but they were mostly so.

SUMMARY

The studies here reported pertain to infection of the wheat plant by *Ophiobolus graminis* Sacc., the cause of the disease known as take-all. It has been shown which of the tissues studied are invaded and what are the morphological and chemical changes produced by such invasion. The microchemical studies here reported were made on the roots only.

The behavior of the primary and secondary roots in the presence of the parasite is very similar. The fungus penetrates the epidermis, passes through the cortex, and enters the stele. Its progress is hindered to some extent by the endodermis.

Many of the cell walls with which the parasite comes in contact become thickened, or the walls may produce elongated protuberances in front of and around the invading hyphae. These previously undescribed structures are given the name lignitubers.

All the cells and vessels of the stele, except the xylem tubes and some cells near it, may be distintegrated after penetration.

Microchemical studies made on diseased roots showed that the disease caused a nonuniform reduction of cellulose, which was replaced by lignin and a slight amount of suberin. This is especially true of the thickened cell walls and the lignitubers. Other substances are not changed by the disease.

The coleoptile is penetrated through the unbroken epidermis, which is greatly thickened, and many lignitubers are formed in its cells. During the progress of the hyphae inward the mesophyll is destroyed except that near the bundles. The inner epidermis stops the further progress of the fungus and thus the coleoptile protects the young seedling, at least to some extent.

The subcoronal internode is attacked through the epidermis and may become infected in all parts. The pith and the phloem may become disintegrated. The xylem tubes and cells in their vicinity respond to the presence of the organism by thickenings on their inner walls by which they may be filled completely.

Studies of the crown were made on plants that had recovered from the disease. In these plants the lower part of the crown and the lower secondary roots were found to be injured. The hyphae had entered the crown through the pith and xylem tubes of the subcoronal internode and the xylem of the lower secondary roots. In many cases the xylem tubes where they enter the crown were plugged by excessive thickenings on their inner walls.

The results here reported have been found useful in interpreting the behavior of wheat plants affected with take-all and may have a bearing on the explanation of resistance, if resistant plants are found.

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THE INOCULATION OF PACIFIC NORTHWESTERN RIBES WITH CRONARTIUM RIBICOLA AND C. OCCIDENTALE[1]

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INTRODUCTION

The native piñon blister rust or nut-pine rust (*Cronartium occidentale* Hedgc., Beth., and Hunt) (*11*),[3] which has been known in Colorado for many years on species of Grossulariaceae, is now known to extend westward into southern California and northward into the inland regions along the Nevada-California boundary and into southern Idaho. One collection of this rust was made as far north as Spokane, Wash., on *Ribes aureum* Pursh, in 1914 (collection No. 6360 by W. E. Flowers; FP No. 41916[4]). Baxter (*1*) found the rust in northern Wyoming south of Buffalo. *C. occidentale*, although widely distributed, has little economic importance.

The white-pine blister rust (*Cronartium ribicola* Fisch.), which is believed to have been introduced into the Pacific Northwest on a single importation of eastern white pines from France (*2*), like the piñon rust, occurs on species of Grossulariaceae, but its alternate stage attacks 5-needled pines. This rust is now thoroughly established in British Columbia and has been found at numerous points in both eastern and western Washington (*19*)[5] and in Oregon. It is known to exist in one county in Idaho. Undoubtedly it will continue to spread eastward and southward. This rust has shown itself capable of causing great damage to *Pinus monticola* D. Don., the species on which forestry in northern Idaho mainly depends. The present encroachment upon the forest areas of this region by the white-pine rust is of the gravest concern to the future of forestry in Idaho and should be the immediate signal for localized control work.

Advance infections of both rusts are found in their uredinial stages on species of the Grossulariaceae, which for convenience in this paper will be usually grouped under the name Ribes. A method is therefore needed by which any Cronartium found on Ribes in Idaho can be definitely assigned to the proper species. When *Cronartium ribicola* begins to invade the range of *Pinus lambertiana* Dougl. in California, a similar need will be felt there. The rusts are morphologically quite distinct on pines (*6*), but their stages on Ribes can not be certainly distinguished by any morphological criteria so far developed. Hedgcock's observations in the field and in the green-

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[2] The writer is under obligation to members of the Washington staff of the Office of Forest Pathology and to R. H. Colley, formerly of that office, for advice and assistance in conducting the investigation; to the members of the Office of Blister-Rust Control of the Bureau of Plant Industry and to E. P. Meinecke, of the Office of Forest Pathology, and J. S. Boyce, formerly of the latter office, for their cooperation in procuring Ribes and rust material for inoculation purposes; and to Carl Hartley, of the Office of Forest Pathology, for his valuable assistance in the preparation of the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 681.

[4] Specimen collection number of pathological specimens for study, Office of Forest Pathology.

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house show that the telial columns of *C. occidentale* (*11*) are somewhat darker colored (walnut brown to Vandyke brown (*20*)) than the Sanford's brown ones of *C. ribicola*, and the average length and the thickness of wall of its urediniospores are significantly greater (*5*). Some of the Cronartium specimens, however, are so near the border line separating the two species that their identification by any of these characters becomes extremely dubious.

Because of the situation just described, an investigation was undertaken to differentiate physiologically the uredinial stages of the two Cronartiums by studying in the greenhouse their reactions on various species of Ribes. With this end in view, an extensive collection of native and cultivated Grossulariaceae, representative of those found in different parts of the United States, Canada, and Europe, were propagated at the pathological greenhouses of the United States Department of Agriculture, Washington, D. C. When these plants became available for inoculation purposes they were inoculated with the two blister rusts under similar conditions. For these comparative inoculations environmental conditions were maintained as favorable as possible for rust infection and development. The results given in this paper are those obtained from the inoculation of Ribes hosts from the Pacific Northwest, which are potentially hosts of these two blister rusts.

VALUE OF GREENHOUSE INOCULATION DATA

Results with *Cronartium ribicola* on the Pacific Northwest Ribes are of especial value in indicating the possibilities of the spread of this introduced disease in that region. While greenhouse inoculation results do not always tell the same story as field tests, because of the uncertainty and extreme variability of field conditions, nevertheless the former are of particular value. As a general rule, when experimental field tests with rusts have been supplemented by greenhouse tests, it has been found that the artificial inoculation results obtained in the greenhouse indicate with considerable accuracy what may be expected to occur in the field when a given host species or variety is exposed to infection.

The importance of agreement between greenhouse and field conditions has been pointed out by other rust investigators. According to Spaulding (*21*), the estimates for the inside inoculation experiments with *C. ribicola* on Ribes species and varieties agree surprisingly with those made entirely independently out of doors. Fromme and Wingard (*10*), in their investigations of the varietal susceptibility of beans to rust, also give evidence that greenhouse tests that have supplemented field tests form a reliable index of the field behavior of a variety with respect to rust infection. Melchers and Parker (*15*) in a comparison of nursery and greenhouse results agree with them, for they report that their results with wheat rust in the greenhouse were similar to those produced under field conditions in the rust nursery where conditions were as severe as those to which a commercial field is subjected in a natural epidemic. While field tests must finally decide the susceptibility of any species or variety of Ribes to either piñon or white-pine blister rust, the greenhouse results give an excellent indication of what may be expected in the field under conditions favorable for infection.

When a Ribes species proves fully susceptible in the greenhouse under controlled conditions but produces a reduced number of urediniospores in the field, this difference can usually be explained either by field conditions unfavorable to infection or by the growth habits of the host; i. e., the time and manner of leaf production, or the distance from infected white pines. Disagreements would, therefore, be expected in comparing the order of relative susceptibilty of Ribes species in the greenhouse and in the field.

SOURCE OF THE INOCULUM

Cultures of the two Cronartiums were obtained from the field from representative localities. No attempt was made to procure single-spore lines. For the purposes of the experiments a mixture of lines from the same locality was not regarded as objectionable. As has been stated above, the problem was chiefly the differentiation of the two species of Cronartium on Grossulariaceae, which are heterozygous hosts. Evidences of geographic strain differences for each of the two species were, however, also observed.

Strains of *Cronartium ribicola* were obtained from New England and from British Columbia in the Pacific Northwest, and in 1922 a strain from Scotland was also secured. Strains of *C. occidentale* were obtained from Colorado, southern California, Nevada, and Wyoming.

BEHAVIOR OF THE BLISTER RUSTS IN GREENHOUSE INOCULATION

The best urediniospore infection was generally secured during the late summer and fall upon leaves that had fully expanded but had not commenced to harden. Stakman and Piemeisel (*23*) recorded *Puccinia graminis* as developing unusually well in late September and early October, a period that they found ideal for rust development in the greenhouse. Spaulding (*21*) likewise observed that leaves produced by buds developing in late summer or fall, even if very small, readily become infected. High temperatures in the late spring, summer, and early autumn interfered seriously with successful inoculations. At the beginning of cooler weather, during the autumn period, the cultures kept over the summer in the greenhouse revived vigorously upon congenial Ribes hosts.

Uredinia of both *Cronartium ribicola* and *C. occidentale* generally appeared within 8 to 14 days. A large number of comparative inoculation experiments conducted for the purpose of determining the incubation period of uredinia under the same inoculation and infection conditions showed no essential difference between the two species. The average length of time between the actual inoculation of Ribes and the production of mature urediniospores was 11 days. Uredinial development was most vigorous during the bright, sunny weather of spring and early fall; cloudy weather retarded rust development, appreciably delaying the appearance of uredinia, especially during the winter months. Under favorable conditions abundant stock cultures of strains of both rust species, systematically maintained for the purpose of supplying spores for inoculating purposes, could be readily procured within approximately the same period of time upon congenial species of the *Ribes aureum* group (*R. aureum*, *R. odoratum* Wendl., and *R. gracillimum* Cov. and Brit.) or upon certain varieties of *R. nigrum* L. Such stock cultures persisted in a good condition for approximately three weeks, when the most heavily infected leaves began to dry up and drop from the host plant. A successful maintenance of vigorous stock-culture material was largely dependent upon attendant weather conditions. Artificial inoculations proved to be quite necessary in order to insure the continuous propagation of the different strains, because self-inoculation could not be depended upon. Even on heavily infected plants leaves that were uninfected rarely became infected as a result of self-inoculation.

A moderate temperature up to 75° F. (24° C.), the temperature at which Ribes plants make their best development in the greenhouse, was found to be most favorable for uredinial development of the two Cronartiums. This is coincident with the finding of Stakman and Piemeisel (*23*) for *Puccinia graminis*. Mains (*14*) states that a temperature of 16° to 20° C. is most favorable for the germination of rust spores, few germinating below 5° or above 30°. Peltier (*18*) gives 20° C. as the optimum temperature for infection and subsequent development of stem rust. According to Doran (*7*), the minimum, optimum, and maximum temperatures for the germination of urediniospores of *Cronartium ribicola* are 8°, 14°, and 25° C., respectively. During exceptionally warm weather in the greenhouse under conditions of high temperatures, congenial plants, ordinarily heavily infected, were observed to show only a very much reduced number of uredinia. Pustules that developed under such conditions might become aborted or otherwise abnormal.

To insure maximum rust infection an atmosphere close to saturation was necessary, and for normal development a considerable amount of sunlight was needed. Peltier (*18*) has observed that where other environmental factors are equal, the duration of the period of sunshine is more important than its intensity in influencing the production of rust infection.

The rusts infect unhardened leaves most readily and vigorously. Stakman and Piemeisel's best infection results with *Puccinia graminis* (*23*) were generally obtained on young leaves inoculated at an early stage of development, although in the case of some grasses the older leaves became infected more readily. Evidence that the older Ribes leaf is more susceptible to infection from *Cronartium occidentale* has also been obtained. The data will be presented in another paper.[6]

As a general rule matured senescent leaves produced fewer uredinia and favored the development and production of telia. Telia developed on Ribes when metabolism and growth were apparently slowed up. Mains (*14*) states that this same condition exists in the case of *Puccinia triticina*, which he has never observed to produce telia except on old leaves when the wheat plants approached maturity. Matured leaves of certain Ribes species bore neither uredinia nor telia and were quite immune to infection. In midwinter it was a common experience to secure on young leaves of the *Ribes aureum* group mostly uredinia of both rust species. For both Cronartiums a relationship is thereby indicated not only between the age of the leaf of the Ribes host and the type of spore produced, but also between the age of the leaf and the degree of receptivity. Spaulding (*21*) has already stated the above-mentioned facts for *C. ribicola*. Kroemer (*13*) in his investigations of the disease of grape leaves caused by *Plasmopara viticola* reports a somewhat similar connection between the age of the leaves and their liability to infection. Under the condition of artificial inoculation he found that young leaves were more vigorously attacked than old ones and that very young leaves completely resisted every attempt at infection.

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[6] HAHN, G. G. A PHYSIOLOGICAL METHOD OF DISTINGUISHING CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE IN THE UREDINIAL STAGE. [Unpublished manuscript.]

Observations on greenhouse cultures of both *Cronartium ribicola* and *C. occidentale* showed that they remained viable under favorable conditions for 30 days or more. Other investigators, including Fromme (*9*), Melhus and Durrell (*17*), Doran (*8*), and Peltier (*18*), have shown that the urediniospores of a number of rust species may remain viable for a month or more. Spaulding (*21*) gave the longevity of urediniospores of *C. ribicola* as 7 to 270 days. When inoculations were made under conditions favorable for infection and negative results were obtained, particularly on congenial hosts, there is a possibility that some aberrant condition in the formation or maturation of the urediniospores had interfered with their ability to germinate. A few germination tests with different lots of inoculum indicated that variation in germination does exist. It seems even more likely, however, that some temporary physiological condition of the host existing at the time of inoculation might inhibit infection. This was found to be the case for certain host species during the inoculation experiments when the spores used were known to be viable. As these same plants were successfully inoculated later, the second explanation seems to be the more probable.

METHODS

In general the methods employed in the greenhouse inoculation were in line with those developed by Stakman and his associates (*22*, *23*, *24*), working with cereal rusts, and by Spaulding and his associates (*21*) in the white-pine blister-rust inoculations. Because of cultural differences between the Ribes hosts and such hosts as cereals and grasses, certain necessary modifications in methods were made.

CARE OF RIBES PLANTS TO BE INOCULATED

For the inoculation experiments it was found necessary to insure the production of freshly developed, fully expanded, and unhardened leaves. The cereals and grasses worked upon by other rust investigators may be propagated from seed at any time and are therefore available for inoculation within approximately three weeks. Ribes are not so readily propagated. For these investigations plants of Ribes were secured by the quickest means possible, namely, by growing the cuttings of the vigorous growth of the current season, which were secured in the fall, and by removing plants from the field to the greenhouse where they were grown in pots. In the former case rooted cuttings were usually not available for inoculation purposes until the next year, whereas transplants from the field were frequently usable soon after they were transferred to the greenhouse. In procuring field transplants the tops and roots were cut back, care being taken not to allow the roots to become dry. Shipment was made in slightly moistened moss wrapped in oiled paper. As soon as the plants were received they were potted in good greenhouse soil and placed for a time in a cool situation. Such plants broke into leaf soon after potting, depending, of course, upon the species and the particular adaptiveness of each to such treatment. As a general rule most of the Ribes species responded readily to this treatment.

Ribes plants secured from cuttings varied in that those of some species produced more than one set of leaves for inoculation during the year, and those of others produced only one set of leaves during the season. It was not always possible to secure leaves for inoculation purposes at a specified time. Leaves in condition for inoculation were utilized as they were produced, viable inoculum being provided for the test made under as favorable environmental conditions as possible. Test plants bore metal labels giving not only the name of the host species, but also its source and the date of acquisition. Additional labels gave the data for each inoculation (the date, the species, and the collection number of the fungus strain).

In these investigations it was not possible to discard plants after they had been used once for inoculation purposes, particularly if the plant happened to be a rare species or one difficult to procure or grow. Instead, such plants were stripped of all leaves after results had been recorded, dipped in a corrosive sublimate solution 1:1,000, and set aside in a section of the greenhouse apart from the culture chambers where they could be watched until new leaves appeared. Stock plants for the propagation of inoculum of the different strains were particularly watched for any possible appearance of the rust upon them, but such unintended infections were extremely rare. When the plants were in condition to be reinoculated they were used as hosts for the same rust culture with which they had been previously inoculated. This procedure safeguarded the purity of individual culture strains of the two Cronartiums.

Continual stripping gradually weakened certain of the plants, particularly those difficult to grow in the greenhouse and those with scanty root systems, but vigorous growers such as plants of the *Ribes aureum* group and *R. nigrum* have been defoliated again and again without much apparent injury.

The present investigations were concerned largely with allied species and varieties of the common garden currant, *Ribes sativum* (*R. vulgare*). Inasmuch as this particular host group normally produces only one set of leaves in the spring at the time of fruiting, it became necessary to find a method whereby inoculable leaves could be procured throughout the year, and especially during the late summer and autumn. Plants placed in cold storage before the spring burst, could upon later withdrawal be relied upon to furnish inoculable leaves during the early part of the summer as well as in the late summer and fall, when rust specimens on Ribes from the field are most likely to be sent in for identification.

When plants were not being used in the inoculation experiments they were isolated in sections of the greenhouse where rust cultures were not present, or kept out of doors and brought into the greenhouse at a later date when required. Care was exercised in going from the sections of the house containing plants infected with rust to the rust-free plant sections, so that infection would not be carried over into the latter. To guard against such an occurrence the rust-free plant sections were isolated in inclosed portions of the greenhouse; cloth dusters, which were removed before leaving, were worn in the infected sections, and care was taken at all times to wash the hands thoroughly before handling uninfected plants, particularly after working about rusted ones. Whenever possible, care was exercised in excluding insects from the greenhouse. Inoculations were never made with the two blister-rust species on the same day. Buffer uninoculated plants of congenial species were maintained on a shelf 5 feet high down the center of the house between the stock cultures on one side bench and the experimental plant inoculation series on the opposite side bench. Throughout the experiments the plants kept on this shelf did not become infected.

MAKING INOCULATIONS

In general, plants were inoculated with spores applied by gently pressing uninfected Ribes leaves that previously had been finely sprayed with water from an atomizer against leaves well covered with fertile spores. Tap water was used with satisfactory results, although Melhus and Durrell (*17*) state that it may have a toxic effect on the germination of certain kinds of rust spores. Recently produced urediniospores were used, as such material usually could be depended upon to germinate to a certain extent. Viable spores were bright orange yellow in color. Care was taken to keep the inoculum from becoming contaminated by mildew or other fungi, which frequently infect greenhouse rust cultures and cause infected areas to die out rapidly, at the same time apparently affecting the viability of the urediniospores themselves. Mildew particularly was troublesome at times, especially in the early spring when the temperature remained low and the sky was overcast. It was kept down to a minimum, however, by picking off and destroying all infected leaves, including those showing the barest trace of mildew. Mildewed leaves rarely became infected, and their inclusion in the inoculations only tended to increase the spread of the contamination.

Plants were also inoculated in the manner described by Carleton (*3*) and Fromme (*9*), that is, by dusting spores from heavy infections over uninfected plants that had been previously sprayed. When the inoculum was very limited, spores were frequently applied to the atomized leaves by means of a scalpel. Extreme care was necessary in this latter process, particularly in the case of very delicate leaves, lest the leaf tissues be injured or killed. Throughout the tests an abundance of inoculating material was highly desirable, in order to insure a general distribution of spores over the leaf surface and maximum infection. Negative inoculation results were confirmed wherever possible by repeating the tests with the same plants and the same rust strains.

TIME OF INOCULATION

Inoculation tests were confined largely to the early spring months, late summer, and autumn. Very little experimental work was conducted during the summer when high temperatures prevailed. However, stock cultures were maintained throughout the high-temperature period. During the summer the glass roof of the greenhouse was lightly whitewashed to cut down the intensity of the sunlight. Iceless refrigerators of the type devised by Hunt (*12*) were used for inoculation, the plants being kept in these moist chambers for 48 hours. During the very warm summer period the inoculation chambers were installed out of doors on the north side of the greenhouse beneath the trees, where the maximum temperature did not exceed 85° F. A continuous flow of water seeping downward through the cloth sides from the water bath on top of the inoculation chamber kept the plants within at a reduced temperature because of the constant evaporation of water from the surfaces of the wet curtains. As in the greenhouse inoculations, the plants were finely sprayed before they were placed within the iceless refrigerator. This film of moisture on the Ribes leaves was constantly maintained while they remained within the inoculation chamber by turning the stream of water from a garden hose quickly and lightly at intervals against the cheesecloth curtains and forcing the water through in a fine spray. Plants inoculated in extremely hot weather were kept out of doors for some days after they were taken from the iceless refrigerator. When pustules began to form the plants were immediately returned to the greenhouse in order to prevent the possible infection of any Ribes that might be in the neighborhood.

CARRYING STOCK CULTURES

Stock cultures of the two Cronartium species were kept in separate compartments of the greenhouse. Partitions of a double thickness of cheesecloth were used in each compartment to separate the side benches into culture chambers in which the individual strains were kept isolated. The cloth partitions extended to the height of the side wall of the house after the manner described by Stakman, Piemeisel, and Levine (*24*). The benches on which the rusted plants stood were kept well drained and fairly dry by covering the greenhouse benches with sifted cinders. An abundant supply of vigorous inoculum was maintained by making new stock cultures approximately every two weeks. The different generations were kept separate. The rusted plants were, moreover, watered only at the base, thereby reducing possible chances for self-inoculation. Generations were indicated on the pot labels by supplementing the original collection number given the strain with abbreviated symbols of the hosts in inoculation sequence. These host abbreviations in turn were supplemented with subnumbers indicating the number of inoculations of each particular Ribes species, i. e., "II *C. ribicola* BC, 38805 nig ^{7} au ^{1}" signifies seven successive generations of uredinia of the British Columbia strain of *Cronartium ribicola* on *Ribes nigrum* followed by one generation on *R. aureum*. A record of the inoculation data of successive generations was kept for future reference in explaining possible variations in the reactions of a given rust strain, particularly with regard to possible decline in vigor.

COMPARATIVE STUDY OF THE TWO CRONARTIUMS

In a series of rust-infection tests, two species of Cronartium were used. The inoculations were made as close to each other in point of time as possible, the procedure being to handle only one species of rust on the same day. More than one strain of the same species, however, was sometimes handled on the same day, but the utmost care was taken to avoid mixtures. Comparative inoculations of the two blister rusts were made wherever possible on Ribes plants of the same species, collection source, and leaf age. Wherever possible both rusts were used on plants derived from the same original parent stock. Occasionally the same plant was inoculated with both rusts. One side or branch of the plant was first inoculated with one of the rusts. During this process the rest of the plant was protected against chance inoculation. Two days later the inoculated part was protected and the opposite side or another branch was inoculated with the other rust.

Throughout the tests an effort was made to maintain environmental conditions influencing rust infection as nearly identical as possible for both rust species. As previously pointed out, these conditions included temperature, humidity, and light, as well as the condition of the leaves of the Ribes host at the time of inoculation. Inoculations were made when possible at temperatures of not more than 75° F. A high relative humidity close to the saturation point was maintained throughout the infection period. After the inoculated test plants were removed from the iceless refrigerator they were placed in the muslin-partitioned isolation chambers on the side benches. Ordinary greenhouse watering was sufficient to carry infected plants to the sporulating stage. The amount of sunlight secured in the greenhouse sufficed for excellent rust development. The light conditions obtained throughout the culture and inoculation test chambers were quite comparable. Records were systematically kept of the first appearance of infection spots, pustules, and spores. These records were complete within 15 days, so far as maximum urediniospore production was concerned. A 15-day period was also found to be sufficient for the appearance of those pathologic symptoms that indicated the particular infection type of susceptibility or resistance of the given host species.

RECORDING DATA

The inoculated plants were classified by infection types, based on the pathologic symptoms produced. Where it was necessary because of a scarcity of good trial-host material to inoculate plants not in condition for inoculation, a record was made at the time of such aberrant host conditions in order to aid in the explanation of possible negative results. An attempt to describe individually all the pathologic symptoms for each tested host species and variety, would have required a great deal of time. Classification symbols were therefore developed, the use of which greatly facilitated the recording of results. The symbols indicating the types of infection are as follows:

Resistant types:
- Immune—
  - ○ No uredinia formed; hypersensitive or necrotic areas either definite, indistinct, or entirely lacking.
- Resistant—
  - ◑ Uredinia, formed but minute or aborted, surrounded or associated with hypersensitive flecks or larger necrotic areas; flecks in some cases very abundant.

Susceptible type:
- ● Uredinia, normal size; no hypersensitive or necrotic areas, but in some cases infections surrounded by slightly chlorotic tissue, or uredinia produced on green islands.

The relative quantity or abundance of uredinia produced on the infected leaves was also indicated by symbols. They are as follows:

=, *Trace.*—Uredinia bare trace or very few in number.

—, *Slight infection.*—Number of uredinia below normal, scanty. This symbol is analogous to the single cross, ×, used in former white-pine blister-rust investigations (*21*).

±, *Moderate.*—Medium production of uredinia, average or normal infection. Analogous to the two crosses, ×× (*21*).

+, *Heavy.*—Infection heavier than medium. Analogous to the three crosses, ××× (*21*).

++, *Very heavy.*—Heavy or extremely abundant production of uredinia, covering practically the entire leaf area.

To indicate the number of infected leaves on each plant as related to the number of inoculated leaves, a fraction was used, the denominator giving the number of leaves inoculated and the numerator those showing infection. In recording the abundance of uredinia produced on each host species or variety, a rating was given the plant as a whole.

CORRELATING DATA

To obtain a general figure for the abundance of urediniospores on the uredinia-bearing leaves of a given Ribes species or variety, it seemed best to reduce the symbols described in the preceding section to a numerical basis and average them. Table 1 gives the numerical expression for each symbol.

TABLE 1.—*Mathematical expression of the abundance of uredinia production*

Symbols for relative abundance of uredinia on infected leaves	Equivalent symbols	Range of class [a]	Mid-value of class [a]	Abundance of uredinia
		Per cent	*Per cent*	
=	(×)	Less than 5	2.5	Trace.
−	[b] ×	5–35	20.0	Slight.
±	[b] ××	35–65	50.0	Moderate.
+	[b] ×××	65–85	75.0	Heavy.
+ +		85–100	92.5	Very heavy.

[a] The percentage values are rational expressions of the abundance of uredinia production based on the maximum uredinia development on completely infected leaves of *Ribes nigrum*, under favorable conditions, which were taken as a standard. In converting the symbols into numbers, each was assigned the mid-value of the class it represents.

[b] Used in previous white pine blister-rust investigations. See Spaulding (*21*). Range percentages and values of classes do not apply to these symbols.

In Figures 1 to 3 the class ranges are indicated by horizontal lines. In obtaining the average abundance values shown by the broken line in Figures 1 and 2 and by both lines in Figure 3, the rating for each plant was weighted by the number of spore-producing leaves on the plant.

In Figures 1 and 2 the index of the abundance of uredinia per unit area of all infected leaves for each species is shown by a cross (×) without connecting lines. This was obtained by multiplying the converted mathematical value of the average abundance on uredinia-bearing leaves by the percentage of inoculated leaves that produced fertile uredinia, and dividing the product by 100.

The foregoing classification of infection types and abundance of uredinia for the two rusts, *Cronartium ribicola* and *C. occidentale*, follows that laid down by Vavilov (*25*), who in his investigations of grain rust in the field utilized a classification similar to that employed by Eriksson, which was based on the total production of uredinia and the character and development of the lesions. Stakman and Levine (*22*) adopted a system that is very similar to that of Vavilov. Melhus, Dietz, and Willey (*16*), working with crown rust, *Puccinia coronata*, classified the rust infections they obtained into only three infection types. Their types were arbitrarily chosen to state differences not fixed, but including a certain amount of variation in the degree of infection between the two extremes in each type. The system chosen for the present problem is similar to theirs, in that only three infection types are distinguished, but it differs in that the production of uredinia is considered as a separate though closely associated criterion. The infections were not separated into as many types as were employed by Stakman and Levine (*22*), for the reason that good division points could not be obtained for so many. With the system employed there was rarely any serious doubt as to the class to which an infection should be assigned. The use of narrower and more numerous classes would have made assignment difficult.

The recognition of small differences in reactions of the Ribes hosts to the parasite is more difficult than with the grain rusts, both because there are so few distinguishable types on Ribes and because of the heterogeneity of the host material. The grain and bean hosts on which comparisons have been made have been mainly

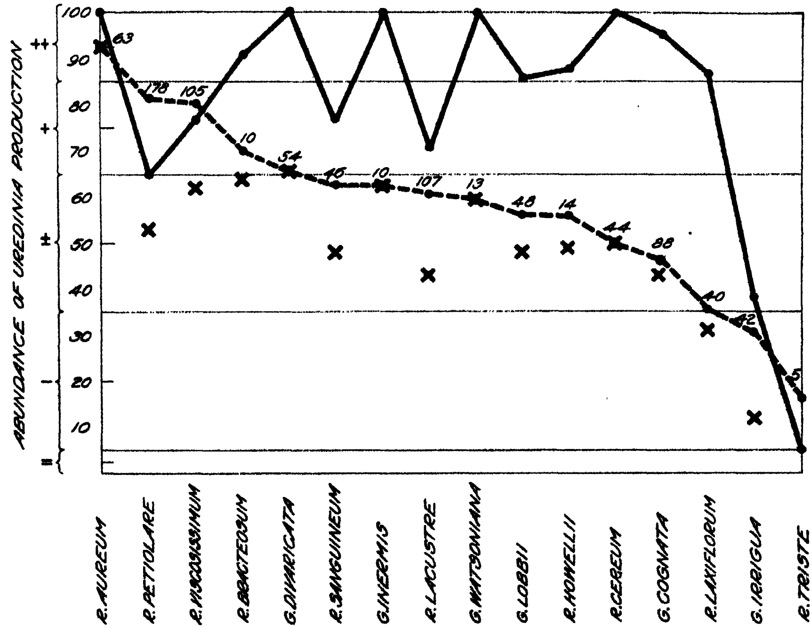

FIG. 1.—Results of inoculations with *Cronartium ribicola* on Ribes and Grossularia plants received from the Pacific Northwest. Only the converted mathematical value of the average abundance of uredinia produced on leaves on which at least some fruiting occurred is considered. Solid line: Percentage of inoculated leaves that produced fertile uredinia. Broken line: The average abundance of uredinia per unit area of leaves on which at least some fertile uredinia were formed. The figures above the points show the total number of such leaves. The symbols at the left margin and their conversion to an arithmetical basis is explained on p. 671. × (points not connected by lines): An index of the abundance of uredinia produced per unit area of leaf inoculated; obtained as described on p. 672

pure lines. The Ribes material from the Northwest consisted of native plants, which are presumably highly heterozygous.

Wherever possible, abundant specimens, which will be permanently available for reference, were collected for the herbarium to supplement the notes taken. When dried quickly under moderate pressure these specimens did not lose much in the way of color or other characteristics.

HOST PLANTS TESTED

The geographical group of native Grossulariaceae treated in this paper, namely, a Pacific northwestern group, which has been classified by Wyckoff as properly belonging in a key for Ribes of Washington,[7] is of particular interest at this time, in that it involves species of Ribes and Grossularia that occur in the regions of advance infections of the introduced *Cronartium ribicola* in the Northwest and which are or may be native hosts of *C. occidentale*. The group includes: *R. bracteosum* Dougl.; *R. petiolare* Dougl. (*R. hudsonianum petiolare* Jancz.); *R. howellii* Greene; (*R. acerifolium* Howell); *R. sanguineum* Pursh; *R. laxiflorum* Pursh; *R. aureum; R. triste* Pall. (*R. ciliosum* Howell; *R. migratorium* Suksd.); *R. cercum* Dougl. (*R. inebrians* Lindl. or *R. reniforme* Nutt.); *R. viscosissimum* Pursh; *R. lacustre* (Pers.) Poir. (*R. parvulum* Rydb.); *R. montigenum* McClatchie (*R. molle* Howell, *R. lentum* Coville and Rose); *Grossularia lobbii* (A. Gray) C. and B. (*R. lobbii* A. Gray); *G. watsoniana* (Koehne) C. and B. (*R. watsonianum* Koehne, *R. ambiguum* S. Wats., in part); *G. cognata* (Greene) C. and B. (*R. cognatum* Greene); *G. irrigua* (Dougl.) C. and B. (*R. irriguum* Dougl., *R. divaricatum irriguum* A. Gray, *R. leucoderme* Heller); *G.*

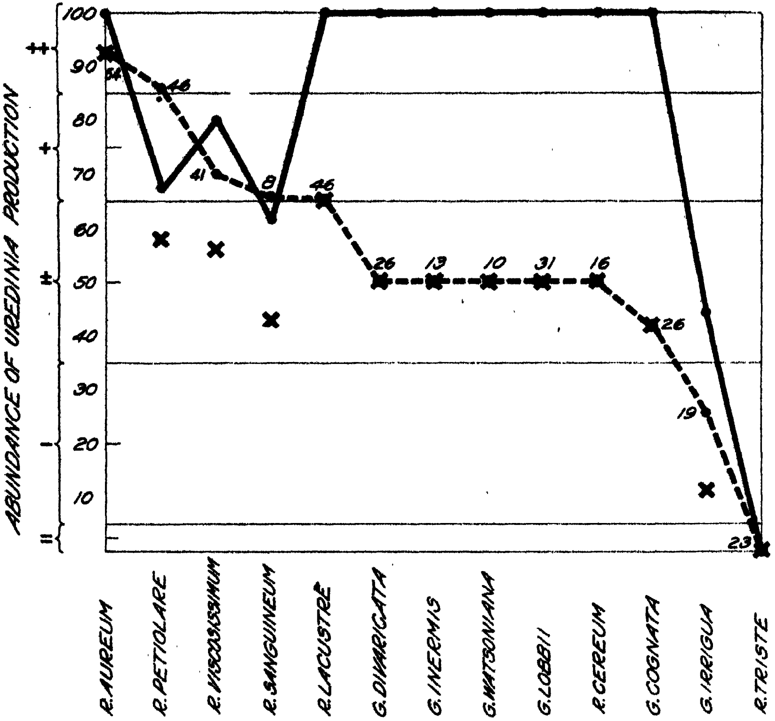

FIG. 2.—Results of inoculations with *Cronartium occidentale* on Ribes and Grossularia plants received from the Pacific Northwest. Only the converted mathematical value of the average abundance of uredinia produced on leaves on which at least some fruiting occurred is considered. Solid line: Percentage of inoculated leaves that produced fertile uredinia. Broken line: The average abundance of uredinia per unit area of leaves on which at least some fertile uredinia were formed. The figures above the points show the total number of such leaves. The symbols at the left margin and their conversion to an arithmetical basis is explained on p. 671. X (points not connected by lines): An index of the abundance of uredinia produced per unit area of leaf inoculated; obtained as described on p. 672

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[7] WYCKOFF, S. N. THE RIBES OF WASHINGTON. 39 p., illus. (U. S. Dept. Agr., Bur. Plant Indus., Off. Blister Rust Control.) 1922. [Mimeographed.]

nivea (Lindl.) Spach. (*R. niveum* Lindl.); *G. divaricata* (Dougl.) C. and B. (*R. divaricatum* Dougl., *R. villosum* Nutt., *R. suksdorfii* Heller); and *G. inermis* (Rydb.) C. and B. (*R. inerme* Rybd., *R. purpusi* Koehne, *R. vallicola* Greene).

All of these species, which were determined in the field, but which did not flower in the greenhouse and thus make further determination possible, were inoculated with *Cronartium ribicola* with the exception of *Ribes montigenum* and *Grossularia nivea*, plants of which were not available for inoculation purposes. For the same reason these two species and *R. bracteosum*, *R. howellii*, and *R. laxiflorum* were not inoculated with *C. occidentale*.

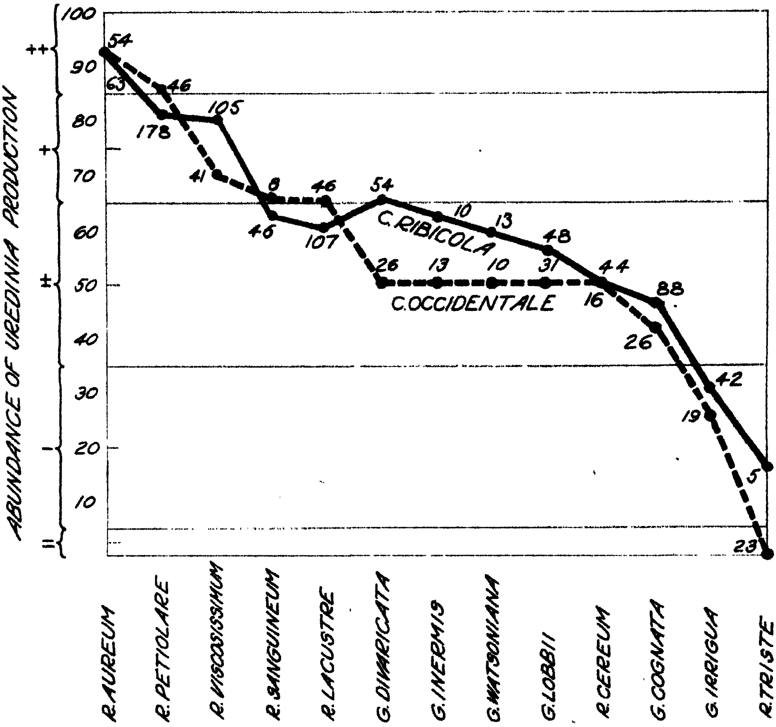

FIG. 3.—Comparison of results of inoculations with *Cronartium occidentale* and *C. ribicola* on Ribes and Grossularia plants as expressed in Figures 1 and 2. Only the average abundance of uredinia on the leaves on which at least some uredinia were formed is considered. The Ribes species that were inoculated with both rusts are included; they are arranged in descending order of the abundance of the uredinia production for *C. occidentale*

RESULTS

All the species of the Ribes and Grossularia tested, irrespective of source, proved susceptible to *Cronartium ribicola*, though some were much less congenial hosts than others. Likewise, practically all proved to be more or less susceptible to *C. occidentale*, except one species, *R. triste*, which was immune. This Ribes species was only slightly susceptible to *C. ribicola*.

TABLE 2.—*Results of inoculating species of Ribes and Grossularia with Cronartium ribicola*

[Types of infection: ○, immune; ◑, resistant; ●, susceptible. Relative abundance of uredinia: =, trace; −, slight; ±, moderate; +, heavy; and ++, very heavy]

Species inoculated		Number of plants inoculated	Leaves inoculated		Number of plants with indicated percentages of inoculated leaves producing uredinia				Number of plants showing on their spore-producing leaves the indicated abundance of uredinia					Number of plants of specified infection type		
Name	Source		Number	Per cent infected	0	1–49	50–99	100	=	−	±	+	++	○	◑	●
Ribes aureum	Idaho (Marble Creek)	6	63	100	0	0	0	6	0	0	0	0	6	0	0	6
Ribes petiolare	Idaho (Elk River) Idaho (Marble Creek) Utah (Salt Lake City)	6 27 4	34 205 36	29 82 0	4 2 4	0 0 0	0 10 0	2 15 0	0 1	0 2	0 3	0 0	2 19	4 2 4	0 3 0	2 22 0
Total		37	275	65	10	0	10	17	1	2	3	0	21	10	3	24
Ribes viscosissimum	Washington (Yakima) Idaho (Hayden Lake) Idaho (Elk River) Idaho (Sandpoint) Idaho (Elbe River)	9 1 6 2 2	51 7 33 28 18	90 86 79 43 83	0 0 2 1 0	1 0 0 0 0	1 1 0 0 2	7 0 4 1 0	1 0 0 0 0	0 0 1 0 1	1 1 0 0 0	0 0 0 0 1	7 0 3 1 0	0 0 2 1 0	0 0 0 0 0	9 1 4 1 2
Total		20	137	77	3	1	4	12	1	2	2	1	11	3	0	17
Ribes bracteosum	Washington (Hoquiam) Washington (Aberdeen)	1 2	5 6	80 100	0 0	0 0	1 0	0 2	0 0	0 0	1 0	0 1	0 1	0 0	0 0	1 2
Total		3	11	91	0	0	1	2	0	0	1	1	1	0	0	3
Grossularia divaricata	British Columbia (Vancouver)	6	54	100	0	0	0	6	0	0	3	3	0	0	0	6
Ribes sanguineum	do	9	60	77	0	3	2	4	2	1	4	0	2	0	2	7
Grossularia inermis	Washington (Yakima County) Idaho	1 1	5 5	100 100	0 0	0 0	0 0	1 1	0 0	0 0	1 0	0 1	0 0	0 0	0 0	1 1
Total		2	10	100	0	0	0	2	0	0	1	1	0	0	0	2
Ribes lacustre	British Columbia (Stanley Park) Idaho (Moscow)	2 17	14 137	50 73	0 3	1 1	0 3	1 10	1 0	1 1	0 6	0 7	0 0	0 3	0 0	2 14
Total		19	151	71	3	2	3	11	1	2	6	7	0	3	0	16

Grossularia watsoniana	Washington (Yakima)	1		100	0	0	0	1	0	0	0	1	0	0	0	1
	Idaho (Moscow)	1	8	100	0	0	0	1	0	0	1	0	0	0	0	1
Total		2	13	100	0	0	0	2	0	0	1	1	0	0	0	2
Grossularia lobbii	British Columbia (Vancouver)	4	56	86	1	0	0	3	0	0	2	1	0	1	0	3
Ribes howellii	Washington (Lake Keechelus)	5	16	88	2	0	0	3	0	0	2	0	1	2	0	3
Ribes cereum	Washington (Spokane)	4	44	100	0	0	0	4	0	0	4	0	0	0	0	4
Grossularia cognata	Idaho (St. Joe)	8	74	100	0	0	0	8	1	0	4	3	0	0	0	8
	Idaho (Sandpoint)	2	18	78	0	0	2	0	1	0	1	0	0	0	0	2
Total		10	92	96	0	0	2	8	2	0	5	3	0	0	0	10
Ribes laxiflorum	Washington (Yakima)	1	3	100	0	0	0	1	0	0	1	0	0	0	0	1
	Washington (Aberdeen)	8	43	86	0	0	4	4	1	3	4	0	0	0	0	8
Total		9	46	87	0	0	4	5	1	3	5	0	0	0	0	9
Grossularia irrigua	Idaho (Oxford)	9	71	13	5	3	1	0	1	3	0	0	0	5	4	0
	Idaho (Moscow)	7	38	87	1	0	1	5	0	3	3	0	0	1	3	3
Total		16	109	39	6	3	2	5	1	6	3	0	0	6	7	3
Ribes triste	British Columbia (Vancouver)	2	3	0	2	0	0	0	1	1	0	0	0	2	0	0
	Idaho (Moscow)	16	71	7	14	1	0	1						14	0	2
	Washington (Yakima)	7	19	0	7	0	0	0						7	0	0
Total		25	93	5	23	1	0	1	1	1	0	0	0	23	0	2

TABLE 3.—*Results of inoculating species of Ribes and Grossularia with Cronartium occidentale*

[Types of infection: ○, immune; ◐, resistant; ●, susceptible. Relative abundance of uredinia: =, trace; −, slight; ±, moderate; +, heavy; and + +, very heavy]

Species inoculated		Number of plants inoculated	Leaves inoculated		Number of plants with indicated percentages of inoculated leaves producing uredinia				Number of plants showing on their spore-producing leaves the indicated abundance of uredinia					Number of plants of specified infection type		
Name	Source		Number	Per cent infected	0	1-49	50-99	100	=	−	±	+	+ +	○	◐	●
Ribes aureum	Idaho (Marble Creek)	5	54	100	0	0	0	5	0	0	0	0	5	0	0	5
Ribes petiolare	do	10	58	79	2	0	2	6	0	0	1	1	6	2	0	8
	Utah (Salt Lake City)	1	10	0	1	0	0	0						1	0	0
Total		11	68	68	3	0	2	6	0	0	1	1	6	3	0	8
Ribes viscosissimum	British Columbia (Vancouver)	1	7	100	0	0	0	1	0	0	0	0	1	0	0	1
	Oregon (Mount Hood)	2	11	82	0	0	1	1	0	0	1	0	1	0	0	2
	Idaho (Hayden Lake)	1	4	100	0	0	0	1	0	0	1	0	0	0	0	1
	Idaho (Elk River)	2	13	100	0	0	0	2	0	0	0	0	2	0	0	2
	Idaho (Sandpoint)	2	16	50	1	0	1	0	0	1	0	0	0	1	1	0
Total		8	51	80	1	0	2	5	0	1	2	0	4	1	1	6
Ribes sanguineum	British Columbia (Vancouver)	3	13	62	0	0	1	2	0	1	1	0	1	0	0	3
Ribes lacustre	Idaho (Moscow)	5	46	100	0	0	0	5	0	0	2	3	0	0	0	5
Grossularia divaricata	British Columbia (Vancouver)	3	26	100	0	0	0	3	0	0	3	0	0	0	0	3
Grossularia inermis	Washington (Yakima)	2	13	100	0	0	0	2	0	0	2	0	0	0	0	2
Grossularia watsoniana	Idaho (Moscow)	1	10	100	0	0	0	1	0	0	1	0	0	0	0	1
Grossularia lobbii	British Columbia (Vancouver)	3	31	100	0	0	0	3	0	0	3	0	0	0	0	3
Ribes cereum	Washington (Spokane)	2	16	100	0	0	0	2	0	0	2	0	0	0	0	2
Grossularia cognata	Idaho (Sandpoint)	4	26	100	0	0	0	4	0	1	3	0	0	0	0	4
Grossularia irrigua	Idaho (Oxford)	5	27	11	4	1	0	0	1	0	0	0	0	4	0	1
	Idaho (Moscow)	4	16	100	0	0	0	4	1	1	2	0	0	0	0	4
Total		9	43	44	4	1	0	4	2	1	2	0	0	4	0	5
Ribes triste	Idaho (Moscow)	5	23	0	5	0	0	0						5	0	0

In the work with the northwestern species 1,230 leaves on 177 plants were inoculated with *Cronartium ribicola*, and 420 leaves on 61 plants with *C. occidentale*. The results of these inoculations are shown in detail in Tables 2 and 3 for the two blister rusts, respectively.

The host material, which is listed in Table 2, does not actually represent the entire distribution of each species from the Pacific Northwest. The representation, however, is to a certain degree indicative for each species; for, despite certain exceptions in species from other regions, it has been the experience of the writer that plants of the same Ribes species from various geographical sources generally react uniformly with respect to rust infection. Some of the plants listed in the table were grown from cuttings received from field men. In such cases it is not known whether each cutting was from a different bush or whether all the cuttings from a particular locality were taken from the same bush. The total number of original plants represented by the inoculated plants is thus probably somewhat less than the total of the figures in column 3 of Table 2.

All the plants inoculated are included in Table 2 irrespective of their leaf condition. Because of scarcity of certain host material it was sometimes necessary to use plants that were not in optimum condition for inoculation purposes. Mildewed or hardened leaves in certain of the tests with *Ribes petiolare*, *R. viscosissimum*, *R. lacustre*, *Grossularia lobbii*, *R. howellii*, and *R. triste* gave negative results.

The results for all the inoculations with *Cronartium occidentale* are given in Table 3. The host species used were obtained and utilized as explained above. To make the comparison between the two Cronartiums as direct as possible, the same plant or plants were regularly used for both rusts in inoculation tests made at different times. Comparisons obtained on identical plants in this way corroborated other results obtained on different plants from the same geographical source. As in the case of *C. ribicola*, no difference was observed in the way in which plants of the same Ribes species but from different parts of the Northwest reacted toward *C. occidentale*.

In the case of very young leaves inoculated just below the terminal or growing-shoot tip, infections of the resistant type were encountered with *Ribes viscosissimum*, *R. petiolare*, *R. howellii*, *R. laxiflorum*, *R. lacustre*, and *R. sanguineum*. Undersized uredinia associated with flecks or larger necrotic areas on the immature leaves of these species gave place to normal uredinia on further developed and fully expanded leaves. On *Grossularia irrigua*, however, a considerable percentage of leaves were either immune or resistant, the latter producing a trace or scant number of aborted pustules associated with flecks and necrosis. This species produced a low percentage of fully susceptible leaves. Leaves of *R. triste* showed a marked tendency to become infected with *Cronartium ribicola* for only a very limited period. Very young leaves produced flecks only, or no signs of infection at all, and fully expanded leaves appeared to become quickly resistant; those that had apparently started to undergo the hardening process were quite immune. Of all the Northwest species handled, *R. triste* appeared to be most difficult to propagate in a healthy condition. This species was particularly susceptible to mildew, which greatly interfered with the results of the rust inoculations.

The different grades of abundance of uredinia production were assigned arithmetical values on a scale of 100, as explained on page 10. The averages of these values for each host species are shown graphically in Figures 1, 2, and 3. In preparing these graphs the results on host material that was not in good condition for inoculation have been eliminated. In reporting the abundance of uredinia production only the infected leaves are considered. The product of this abundance value and the percentage of leaves infected is perhaps a better indication of the amount of uredinia production that might be expected under field conditions. These products for the different Ribes species are shown in Figures 1 and 2 by crosses (×) with no connecting lines.

Some of these greenhouse inoculation results for *Cronartium ribicola* corroborate those already published by Spaulding (*21*). Additional results are given for the first time for native northwestern Ribes which were not available to him, namely, *Ribes petiolare*, *R. howellii*, *R. laxiflorum*, *Grossularia watsoniana*, and *G. cognata*. Artificial inoculations with *C. occidentale* have been previously reported on *R. aureum*, *G. inermis* (*11*), and *G. divaricata* (*4*). All the artificially inoculated hosts of the piñon rust investigated in this paper, other than the three species just named, are therefore reported for the first time.

PACIFIC NORTHWEST RIBES GENERALLY SUSCEPTIBLE TO CRONARTIUM RIBICOLA

The group of northwestern Ribes susceptible to *Cronartium ribicola* presented in Figure 1 are arranged in descending order of abundance of uredinia production. *Ribes aureum* was found generally to harbor a very heavy infection. *R. petiolare*, *R. viscosissimum*, *R. bracteosum*, and *Grossularia divaricata* showed heavy infections. The last-named host was approximately on the class-range boundary line separating the heavy from the moderate infections. On more than half the host species a moderate number of uredinia was produced. Only a scant infection occurred on *G. irrigua* and *R. triste*.

So far as the conclusions from the results of artificial inoculation in the greenhouse can be applied to the field, it is very evident from the data presented in Table 2 and Figure 1 that there is practically no hope of the Idaho white-pine forests escaping the white-pine blister rust because of immunity of the local Ribes species.

PACIFIC NORTHWEST RIBES GENERALLY SUSCEPTIBLE TO CRONARTIUM OCCIDENTALE

The northwestern Ribes group susceptible to *Cronartium occidentale* presented in Figure 2 are arranged in descending order of congeniality to the piñon rust. *Ribes aureum*, a favorite host for *C. occidentale*, was constantly and very heavily infected. Heavy infections were secured on *R. petiolare*, *R. viscosissimum*, and *R. lacustre;* scanty infections were produced on *Grossularia irrigua*; *R. triste* remained quite immune. About half the species belonged in the moderately infected class.

PHYSIOLOGICAL SIMILARITY BETWEEN THE TWO CRONARTIUMS ON NORTHWEST RIBES

From a consideration of Figures 1 and 2 it is very evident that the abundance-of-uredinia production curves for *Cronartium ribicola* and for *C. occidentale* on the northwestern Ribes follow each other rather closely. The parallelism between the results with the two different species of blister rust is indeed very striking, showing how great a physiological similarity with regard to host preference exists between them. This fact, added to their morphological resemblance, impresses on one how slight are the actual differences that exist between these two Cronartiums in the uredinial stage. Figure 3 brings out this physiological similarity between the two blister rusts.

VALUE OF THE NORTHWESTERN SPECIES AS DIFFERENTIAL HOSTS FOR DISTINGUISHING THE TWO RUSTS

From a consideration of the physiological reaction of the two Cronartiums on species of Pacific Northwest Ribes, it is very evident that only one species could possibly be used as a differential host. This species, *R. triste*, proved slightly susceptible to *Cronartium ribicola* and immune to *C. occidentale*. The tests were few and on leaves in poor condition. Because of the difficulties encountered in the propagation and maintenance of this wild host species under greenhouse conditions and its uncertain reaction to *C. ribicola*, it is certainly less useful as a differential host than are a number of the cultivated varieties of its close relative, the common red garden currant, *R. sativum* (*R. vulgare*).

SUMMARY

Species of all the Pacific northwestern Ribes obtainable were inoculated with *Cronartium ribicola* and *C. occidentale* in a greenhouse at Washington, D. C. The methods used in inoculating the Ribes and in determining the results of inoculation are described in detail. The demands of the Ribes host made necessary a considerable modification of the methods used by investigators of cereal rusts in recording and correlating data.

Essential physiological differences were not discovered between the two Cronartiums in the uredinial stage under artificial greenhouse conditions, except in one case. *Ribes triste*, the wild relative of the cultivated *R. sativum* (*R. vulgare*), was immune to *Cronartium occidentale* and susceptible to *C. ribicola*. In these experiments *R. triste* appeared to be a host which was only slightly susceptible to the latter rust, which circumstance, together with difficulties of greenhouse propagation, probably renders this species of little value as a differential host.

Practically all of the northwestern Ribes species proved decidedly susceptible to *Cronartium ribicola*. The inoculation results, taken together with what is known of Ribes distribution in the Northwest eliminate any chance that the *Pinus monticola* forests will escape white-pine blister rust because of the lack of susceptible alternate hosts.

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COMPARISON OF CONFORMATION, ANATOMY, AND SKELETAL STRUCTURE OF A HIGHLY SPECIALIZED DAIRY COW AND A HIGHLY SPECIALIZED BEEF COW[1]

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INTRODUCTION

Dairy type and beef type in cattle are fairly definite. The two types show great contrast outwardly. Much has been written concerning the relative quantity and quality of the edible portions of the carcasses of animals representing the two types, but the differences between their skeletal and anatomical structures have not been clearly brought out.

Because of the emphasis given to the angular, triple wedge shape of the dairy animal and to the blocky form of the beef animal, the tendency has been to imply that the difference between the two types is greater than can be accounted for by the difference in fleshing and that it must extend to the anatomical and skeletal structure of the animal.

A project having for its object the determination of the relationship of the conformation and anatomy of a dairy cow to her milk and butterfat producing capacity has been in progress for some time. Comparative measurements of the external conformation and the size of the internal organs have already been made on a large number of cows. Sophie 19th of Hood Farm, a noted purebred Jersey cow, was presented to the Bureau of Dairy Industry in order that her conformation and anatomy might be studied. This cow, with a production of 17,557.8 pounds of milk and 999.1 pounds of butterfat in one year, held the world's record for the Jersey breed from January 20, 1914, to November 30, 1918. She also had the distinction of having produced 7,544.51 pounds of butterfat in 11 official yearly records. This is the world's record for lifetime butterfat production for Jersey cows. Although this cow did not possess the beauty of type which would have enabled her to win in show-ring competition, her achievements as a producer and as a breeder are sufficient to classify her as an outstanding dairy cow and a representative of the dairy type in its truest sense. Not only was a study made of her conformation and anatomy, but of her skeleton as well.

A need was felt for a comparative study of the conformation, anatomy, and skeletal structure of a cow representing the extreme beef type. For this purpose the purebred Aberdeen Angus cow, Blackbird of Dallas, was obtained. She was selected as a typical representative of this type because she had been a grand champion at the Illinois State Fair and a consistent winner in the show ring for a number of years. She also was a persistent breeder, having produced eight calves. Nothing, however, is known of her ability to produce milk or butterfat, except that she was said to have been a good milker for a beef cow.

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[1] Received for publication Sept. 12, 1928; issued January, 1929.

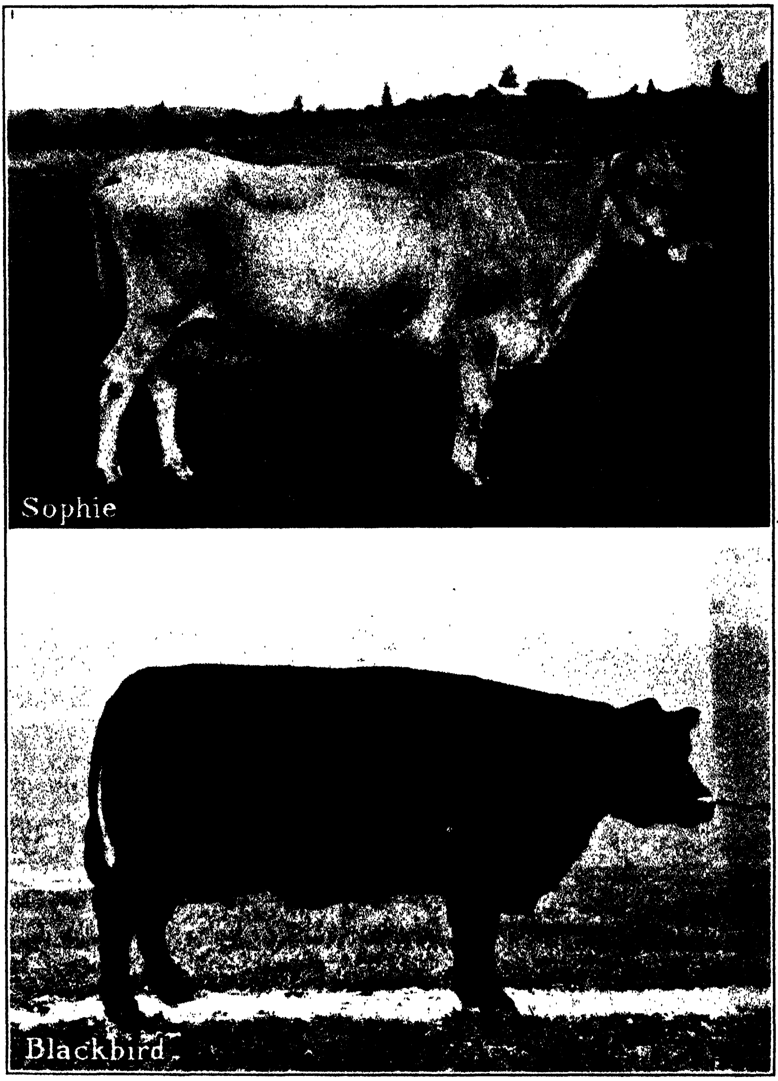

FIG. 1.—Side views of Sophie and Blackbird

For purposes of brevity, these cows will subsequently be referred to as Sophie and Blackbird.

Sophie probably would have been considered a relatively large individual in a breed that, on the basis of weight, is the smallest of all the major breeds of dairy cattle. Blackbird similarly was a

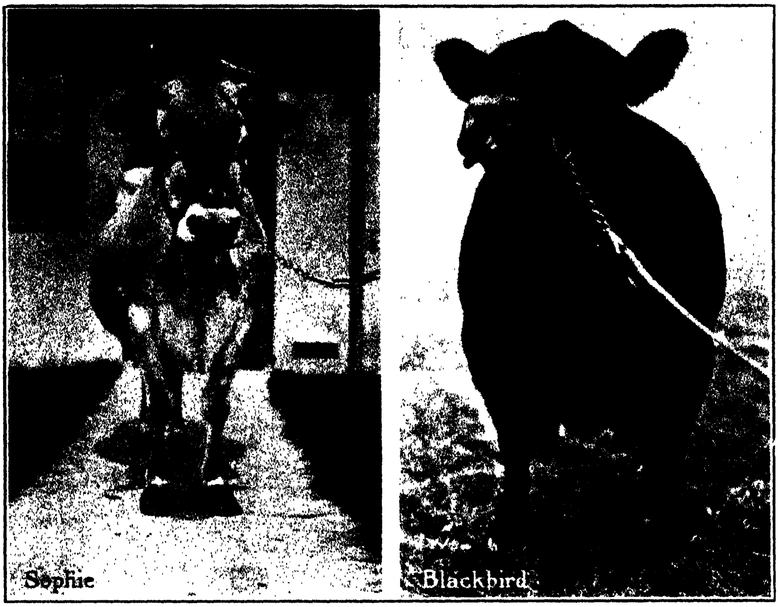

FIG. 2.—Front views of Sophie and Blackbird

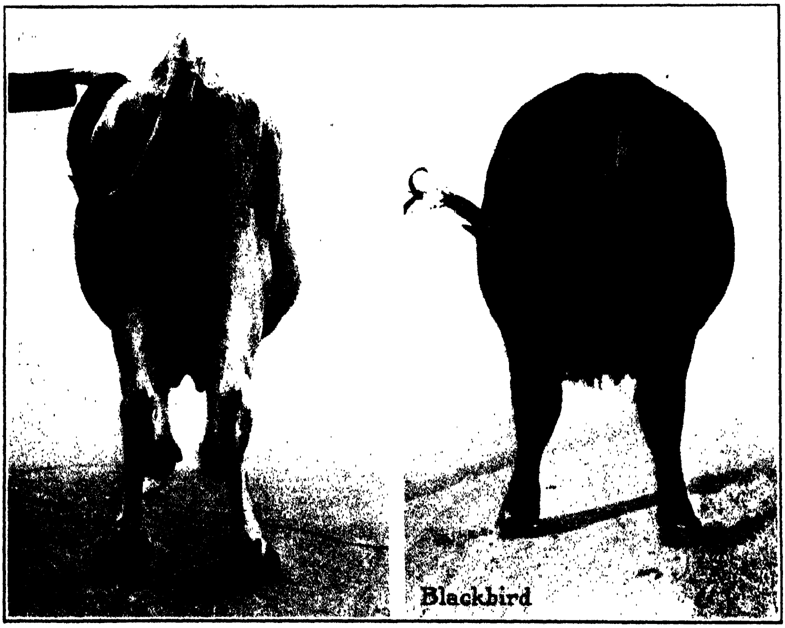

FIG. 3.—Rear views of Sophie and Blackbird

relatively large individual in a beef breed that, in weight, ranks below both the Shorthorn and the Hereford. When obtained, both these cows were well advanced in age. They had ceased to be breeders and had been nonlactating for an extended period. Sophie was dry for 3 or 4 months after a lactation period of 18 months. Blackbird was dry 25 months after a lactation period of 7 months. For comparative purposes these two cows are considered satisfactory to represent the highly specialized dairy type and the highly specialized beef type of cattle. Comparative photographs are shown in Figures 1, 2, and 3.

ANTE-MORTEM DATA

In order to make possible an analysis of the external differences between the two cows, the type of each was translated into numerical values through the application of external body measurements. The plan of measuring was the same as that used by the Bureau of Dairy Industry in studying body development and in determining the relationship of the conformation and anatomy of a dairy cow to her milk and butterfat producing capacity.

Blackbird was in a condition of very high flesh, having been exhibited at the International Livestock Exposition a short time before the measurements were obtained. Difficulty was experienced, therefore, in locating some of the points of anatomy at which measurements were taken. The external measurements representing type or conformation are given in Table 1.

TABLE 1.—*Ante-mortem external measurements of Sophie and Blackbird*

Item	Sophie	Blackbird	Relation of Blackbird's measurements to those of Sophie
			Per cent
Age years	19	12	
Thickness of hide cm	0.66	1.32	200.0
Live weight pounds	927.00	1,565.00	168.8
Height at withers cm	128.17	122.13	95.3
Height at hips cm	122.50	121.71	99.4
Height at pin bones cm	117.00	119.25	101.9
Length, top hips to top pin bones cm	41.00	41.50	101.2
Depth of fore chest cm	71.50	75.00	104.9
Depth of rear chest cm	71.00	78.25	110.2
Depth of paunch cm	71.17	80.25	112.8
Width of fore chest cm	36.17	61.00	168.7
Width of rear chest cm	56.33	65.75	116.0
Width of paunch cm	60.17	73.75	122.6
Width of hips cm	48.00	65.50	136.5
Width of pin bones cm	29.50	34.50	116.9
Width of thurls cm	43.00	52.38	121.8
Width of loin cm	29.00	----------	----------
Length from withers to hips cm	88.50	86.50	97.7
Length from hips to pin bones (tape line) cm	53.00	49.00	92.5
Total length from withers to pin bones cm	141.50	135.50	95.8
Length of loin cm	38.00	----------	----------
Circumference of fore chest cm	177.67	230.00	129.5
Circumference of rear chest cm	203.67	241.50	118.6
Circumference of paunch cm	213.67	261.00	122.2
Width of forehead (tape line) cm	22.00	24.25	110.2
Circumference of muzzle cm	44.00	47.75	108.5
Length from poll to mouth (tape line) cm	56.00	54.50	97.3
Circumference of shin bone cm	16.50	18.75	113.6

As a supplement to the measurements recorded in Table 1, cross-section outlines or contours were made, and a number of values were calculated from the data taken. A discussion of the derivation of these supplementary data will be followed by a general comparative discussion of all the ante-mortem data and of the determinations made on these two cows.

Cross-section outlines or contours of the fore chest and paunch (figs. 4 and 5) were made for both Sophie and Blackbird. They were made by original methods and with specially constructed equipment, and were drawn life-size on specially ruled sheets of paper. The inner curve in each figure is the cross-section outline of the fore chest; the outer curve is the corresponding outline of the paunch. A striking difference in conformation between the two cows is shown by these contours. Coutours are much more significant than caliper measurements of the same body parts because it is possible that two cows may have exactly the same vertical and transverse diameters, yet, because of differences in outline, they may differ widely in cross-section areas. The areas of these contours were measured with a planimeter and are given in Table 2.

TABLE 2.—*Contour areas of fore chest and paunch of Sophie and Blackbird*

Cow	Fore chest	Paunch
	Sq. cm.	*Sq. cm.*
Sophie	1,882	3,226
Blackbird	3,960	4,916

The contour area of the fore chest of Blackbird was 210.4 per cent of that of Sophie, and the contour area of her paunch was 152.4 per cent of that of Sophie. The area of Sophie's fore chest was only 58.3 per cent of the area of her paunch, whereas Blackbird's fore chest area was 80.6 per cent of the area of her paunch.

The following values, which were calculated from the ante-mortem data, are presented to show more completely the relative conformation of the two cows:

(1) The angle of inclination or slope of rump was calculated on the basis of the height at hip, height at pin bone, and the linear distance between them. This angle was 7° 43′ for Sophie and 3° 24′ for Blackbird. The angle was only 44.1 per cent as great for Blackbird as for Sophie.

(2) The thoracic index, which is the relation of depth to width of fore chest, was determined by dividing the former by the latter, giving indexes of 1.977 for Sophie and 1.230 for Blackbird.

(3) The abdominal index, which shows the relation of depth to width of paunch, was determined in a similar manner. Abdominal indexes were 1.183 for Sophie and 1.088 for Blackbird.

(4) A value designed to represent the approximate volume of barrel was determined more or less arbitrarily by multiplying the average of the fore chest and paunch contour areas by the length from withers to line between hips. These two volumes, as determined, were 226,029 c. c. for Sophie and 383,887 c. c. for Blackbird. Blackbird had a volume 169.8 per cent of that of Sophie.

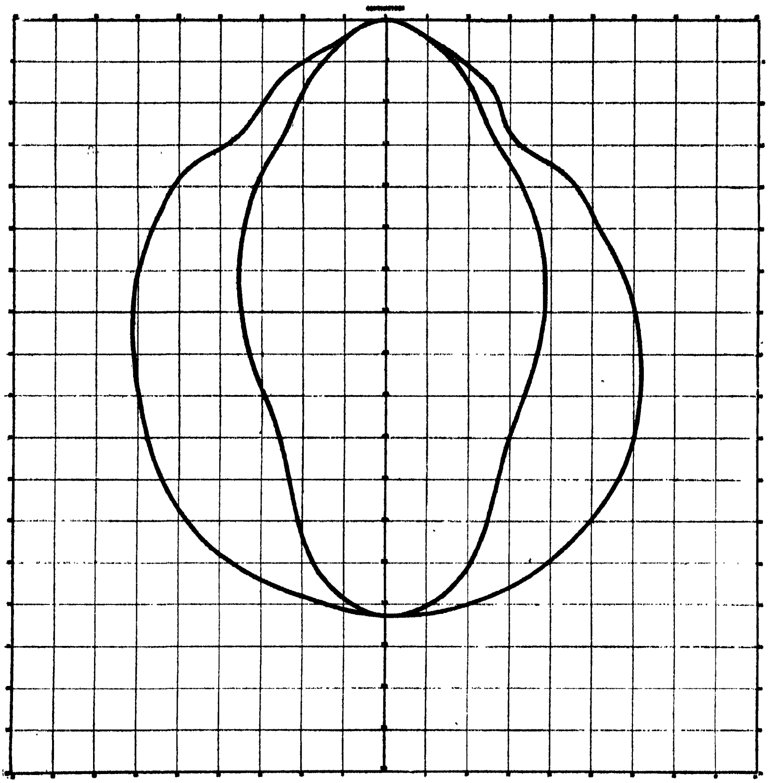

FIG. 4.—Contours of Sophie's fore chest and paunch

(5) The body-surface area was calculated according to the following formula of Hogan and Skouby[2]: $S = W^{.4} \times L^{.6} \times K$, in which S = surface area of body in square centimeters, W = live weight in kilograms, L = length from withers to pin bones in centimeters, and K=217, a constant value for cattle. Since in this comparison the formula was applied to animals of extremely different type and degree of fleshing, there may be some question as to the accuracy of the areas determined, which were 47,460 sq. cm. for Sophie and 57,000 sq. cm. for Blackbird. On this basis the surface area of Blackbird was 120.1 per cent of that of Sophie.

(6) The legginess, or proportion of length of legs to total height, was calculated by subtracting depth of fore chest from height at withers and dividing the difference by the height at withers. Values determined for Sophie and Blackbird were 0.442 and 0.386 respectively.

(7) The relative wedge shape, or difference between paunch and fore chest in depth, width, and circumference, was determined (*a*) in actual units of measurement and (*b*) in the form of a ratio of paunch to fore-chest dimensions. The differences in measurements and the calculated ratios are shown in Table 3.

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[2] HOGAN, A. G., and SKOUBY, C. I. DETERMINATION OF THE SURFACE AREA OF CATTLE AND SWINE. Jour. Agr. Research 25: 419-430, 1923.

TABLE 3.—*Relative wedge shape of Sophie and Blackbird as shown by differences in and ratios of fore-chest and paunch measurements*

Item	Sophie	Blackbird
Differences in measurements:		
Depth of paunch minus depth of fore chest ....cm..	−0. 33	+5. 25
Width of paunch minus width of fore chest ....cm..	+24. 00	+12. 75
Circumference of paunch minus circumference of fore chest ....cm..	+36. 00	+31. 00
Ratios of measurements:		
Depth of paunch to depth of fore chest................	. 995	1. 070
Width of paunch to width of fore chest................	1. 664	1. 209
Circumference of paunch to circumference of fore chest................	1. 203	1. 135

Although Sophie weighed 638 pounds less than Blackbird when ante-mortem data were obtained, she was more than 6 cm. taller at the withers. Blackbird was considerably deeper in body throughout and actually had a far greater vertical wedge shape, as indicated by the increase in depth of rear chest and of paunch over fore chest. Sophie had almost parallel back and belly lines, whereas those of Blackbird were divergent to the extent of more than 5 cm. In width of body, however, Sophie had almost twice as much wedge shape as did Blackbird, but was much narrower throughout. In fact, the ante-mortem measurements, the contours, and the calculated ratios and factors consistently show that Sophie was decidedly

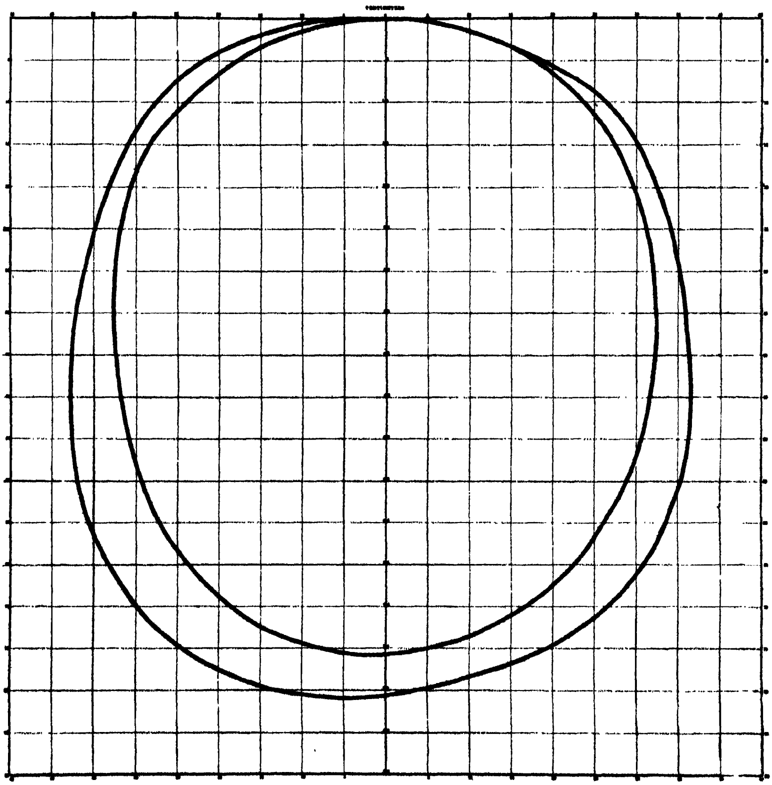

FIG. 5.—Contours of Blackbird's fore chest and paunch

tapering laterally from front to rear but had almost equal depth of fore chest and of paunch. Blackbird, on the contrary, did not taper greatly laterally from front to rear but did show a decided increase in depth of paunch over depth of fore chest.

Measurements from withers to a line between hips and from a line between hips to pin bones taken with a tape line were greater in each case for Sophie than for Blackbird. Sophie, therefore, was not only taller than Blackbird but was also longer in body. The differences in height, however, are relatively slight. Blackbird was 95.3 per cent as tall as Sophie at the withers, 99.4 per cent at the hips, and 101.9 per cent at the pin bones. The average of these three comparative heights shows that Blackbird was 98.8 per cent as tall as Sophie. The total length of Blackbird from withers to pin bones was 95.8 per cent as great as that of Sophie. The external height and length of body of the two cows, therefore, was not strikingly different but was slightly less for Blackbird than for Sophie. The three circumferences of body indicated considerably greater size for Blackbird but a slightly greater wedge shape for Sophie. Blackbird had a greater width of forehead and circumference of muzzle, whereas Sophie had a slightly greater length of head. The shin bone, or metacarpus, appeared to be heavier in the beef cow.

Sophie carried very little body fat. Blackbird, on the contrary, was excessively fat. After she was killed, the layer of subcutaneous fat at the spine in the region of the loin, as nearly as it could be measured, was from 6 to 7 cm. in thickness. The layer of subcutaneous fat at the tenth rib appeared to be from 8 to 9 cm. in thickness. It is obvious that the fleshing may have influenced the different measurements of the beef cow in varying degrees. Although this condition made difficult the location of the hip points and pin bones of Blackbird, it appeared that she was considerably more nearly level in the rump than was Sophie. The fact that the thoracic index of Sophie was much greater than that of Blackbird indicated that in the fore chest she was relatively much deeper in proportion to width than was Blackbird. The abdominal index of the two cows was only slightly different but showed that in the paunch also Sophie was slightly deeper in proportion to her width than was Blackbird. The volume of barrel appeared to be distinctly greater for Blackbird than for Sophie. The body-surface area of Blackbird also appeared to be greater than that of Sophie to the extent of about 20 per cent. The legginess of Sophie appeared to be approximately 15 per cent greater than that of Blackbird.

In Table 1 the only measurements which are lower for Blackbird than for Sophie are the height at withers, height at hips, length from withers to hips, length from hips to pin bones, total length from withers to pin bones, and length from poll to mouth. These signify height of body, length of body, and length of head, all of which may be considered as "scale." Of those values which are greater for Blackbird than for Sophie only five show a difference in excess of 25 per cent. These are thickness of hide, live weight, width of fore chest, width of hips, and circumference of fore chest. It appears that each of these five differences may be largely attributed to Blackbird's heavy fleshing and excessive fat deposition.

POST-MORTEM DATA

In accordance with the plan that has been adopted by this bureau in studying the relationship of conformation and anatomy to producing capacity, the two cows were slaughtered and post-mortem data of both were obtained. Since the skeletons were to be preserved it was necessary that the animals be killed without injury to any bony part.

Sophie was killed by injection of chloroform into the blood stream. Blackbird was killed by bleeding. Sophie's lungs and liver retained large quantities of coagulated blood, and consequently the weight of those organs was excessive. Blackbird's organs when weighed were all practically free of blood. Comparative weight of organs of the two cows, therefore, is not entirely complete. Furthermore, since the skeleton could not be cut or injured, it was impossible to obtain for weighing such organs as the brain and pituitary body. The organs which are available and not affected by retention of blood are listed for comparison in Table 4.

TABLE 4.—*Post-mortem data of Sophie and Blackbird*

Item	Sophie		Blackbird		Relation of Blackbird's data to those of Sophie	
	Weight or measurement of part	Units of weight or measurement per 100 pounds empty body weight	Weight or measurement of part	Units of weight or measurement per 100 pounds empty body weight	Actual units	Units per 100 pounds empty body weight
					Per cent	*Per cent*
Empty body weight ... pounds	768.5		1,476.3		192.1	
Weight of hide ... do	55.00	7.16	82.50	5.59	150.0	78.1
Weight of ovaries ... grams	25.00	3.25	34.40	2.33	137.6	71.7
Weight of pancreas ... do	([a])		371.00	25.13		
Weight of kidneys ... pounds	3.19	.42	3.85	.26	120.7	61.9
Weight of adrenals ... grams	37.00	4.81	57.50	3.89	155.4	80.9
Weight of spleen ... pounds	1.81	.24	1.85	.13	102.2	54.2
Weight of liver ... do	([b])		14.75	1.00		
Weight of small intestine ... do	13.00	1.69	8.70	.59	66.9	34.9
Weight of large intestine ... do	22.50	2.93	7.75	.52	34.4	17.7
Total weight of intestines ... do	35.50	4.62	16.45	1.11	46.3	24.0
Weight of intestinal contents ... do	32.00		19.05		59.5	
Length of small intestine ... feet	132.50	17.24	140.22	9.50	105.8	55.1
Length of large intestine ... do	34.00	4.42	41.49	2.81	122.0	63.6
Total length of intestines ... do	166.50	21.67	181.71	12.31	109.1	56.8
Total weight of empty stomachs ... pounds	47.00	6.12	31.35	2.12	66.7	34.6
Weight of stomach contents ... do	126.50		89.65		70.9	
Total weight of abdominal fat ... do	34.63	4.51	87.15	5.90	251.7	130.8
Weight of right lung ... do	([c])		3.15	.21		
Weight of left lung ... do			2.70	.18		
Total weight of lungs ... do			5.85	.40		
Circumference of heart (near base) ... centimeters	45.00	5.86	43.50	2.95	96.7	50.3
Circumference of heart (over apex) ... do	48.00	6.25	51.75	3.51	107.8	56.2
Weight of heart (trimmed close) ... pounds	3.94	.51	3.90	.26	99.0	51.0
Weight of thoracic fat ... do	5.50	.72	8.75	.59	159.1	81.9
Weight of thyroid ... grams	23.00	2.99	57.50	3.89	250.0	130.1

[a] Pancreas omitted. [b] Liver abnormal. [c] Lungs filled with clotted blood.

Empty body weight is more dependable than live weight, because it eliminates variations due to "fill" of feed or water before weighing or those due to differences in the intervals between feeding or watering and time of weighing. It is determined by subtracting the total weight of contents of stomachs and intestines from the live weight.

The live weights of Sophie and of Blackbird immediately before slaughter were 927 pounds and 1,585 pounds, respectively. Although this difference in live weight between the two cows was only 658 pounds, the difference in empty body weight at time of slaughter was 707.8 pounds.

The number of units of weight or measurement of each organ or part for every 100 pounds of empty body weight has been calculated for both cows to show the relation which each organ or part bears to the total animal structure. These values, in addition to the weight or measurement of the individual organs or parts, are given in Table 4. As one might anticipate, the values for each 100 pounds of empty body weight were found to be relatively much lower for Blackbird than for Sophie. The last two columns in Table 4 show in terms of percentage the relation of the size of organs or parts of Blackbird to those of Sophie, on the basis of (1) the actual units of measure, and (2) the units per 100 pounds of empty body weight.

The determination of the weight of empty intestines is at best subject to some difficulty because of inability to remove all the fat. The length of intestines, on the contrary, is readily determined. The lengths of 166.5 feet for Sophie and 181.71 feet for Blackbird are of interest because of their relative similarity and because these values are intermediate and do not even approach the maximum or the minimum intestine lengths recorded in the post-mortem studies previously referred to.

The lung weights of Sophie were unavailable on account of blood retention. The total lung weight of Blackbird was 5.85 pounds. This is relatively low on the basis of the average of 229 cows slaughtered in a packing house. Although the average empty body weight of these 229 cows was only 941 pounds, the average total lung weight was 7.36 pounds. The weight of Blackbird's lungs was only 0.4 pound per 100 pounds empty body weight, whereas the 229 cows averaged 0.78 pound per 100 pounds empty body weight.

The hearts of Sophie and Blackbird were similar in measurements and almost identical in weight. Each of these hearts, however, was about one-half pound in actual weight below the average weights of 247 hearts obtained in the packing house. Sophie had 0.51 pound, Blackbird 0.26 pound, and the 247 packing-house cows averaged 0.47 pound of heart weight for each 100 pounds empty body weight. The heart weight of Blackbird appears, therefore, to be low in number of pounds and in relation to her empty body weight when compared with data obtained from the 247 cows slaughtered in a packing house.

The only instances in which the actual units of weight or measurement listed in Table 4 are even slightly lower for Blackbird than for Sophie are weight of empty intestines, weight of intestine contents, weight of empty stomachs, weight of stomach contents, and weight and one circumference of heart. As already pointed out, the heart weights and measurements, although differing very slightly, are approximately the same for both cows.

However, since the empty body weight of Blackbird was almost double that of Sophie, the percentage values of units per 100 pounds of empty body weight are approximately half the corresponding percentage values based on actual units of weight or measure, as shown in Table 4. The values in the seventh column range from 17.7 to 130.8 per cent. The table shows also that the weight of body part per 100 pounds of empty body weight is greater for Blackbird than for Sophie in only two instances—total weight of abdominal fat and weight of thyroid.

MAMMARY GLAND

ANTE-MORTEM EXAMINATION

A comparison of the external and internal udder structure of two cows specialized along such widely different lines is of particular interest. Figures 3, 6, and 7 show external views of the udder of each cow. Some of the differences in appearance can be attributed to the season of the year and to the length of the nonlactating period. Sophie was photographed in midsummer, whereas Blackbird was photographed in midwinter, when her coat was heavy and shaggy. As previously stated, Sophie had been nonlactating for 3 or 4 months, whereas Blackbird had been dry for 25 months.

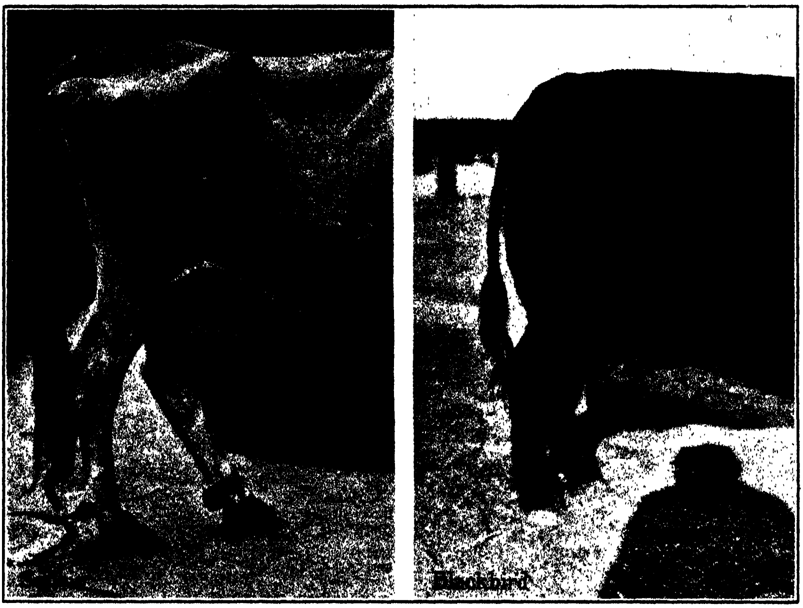

FIG. 6.—Side views of udders of Sophie and Blackbird

Figures 3, 6, and 7 indicate that Sophie's udder was remarkably loose and free of tissue, and that Blackbird's was more compact, more closely attached, and of much greater width and less depth. Detailed observations recorded a short time before the death of each cow showed that Sophie's udder was looser and more yielding and that it contained considerably less total tissue. Blackbird's udder was described as fatty, yet the ante-mortem examination failed to differentiate completely between the fat and the mammary tissue or to indicate the relatively small quantity of mammary tissue in Blackbird's udder as revealed so emphatically in subsequent sectioning of the gland. Ante-mortem examination indicated also that Sophie had less distinctly separated but narrower halves and poorer attachment of gland tissue to abdominal wall. The tissue in Sophie's udder appeared to be much harder and much more coarsely and harshly fibrous than that of Blackbird, which was more stringy. Sophie had greatly superior veins on udder and abdomen and larger milk wells, and the skin covering her udder was thinner, less mellow, and more flexible than that of Blackbird.

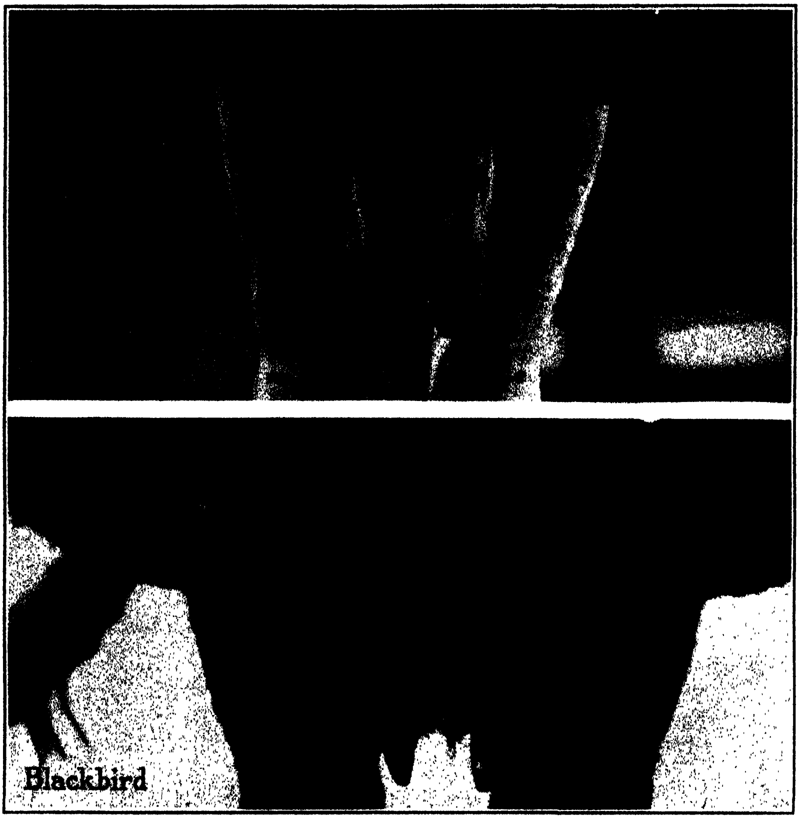

FIG. 7.—Front view of udders of Sophie and Blackbird. These photographs were taken with the camera resting on the ground and the lens directed upward and backward between the forelegs

POST-MORTEM EXAMINATION OF INTERNAL STRUCTURE

In preparing the udder for the study of its gross anatomy, formalin was pumped through the teat canals into the secretory system. Unfortunately, the quantity accommodated by Sophie's udder was not measured, but the average volume of fluid accommodated by seven nonlactating udders of dairy cows has been found to be equivalent to 25.8 pounds of milk. The udder of Blackbird, however, held only the equivalent of 8.22 pounds of milk.

The gross anatomy of the two udders is indicated by Figures 8 to 11, which are of approximately the same relative proportion and which show vertical transverse sections of the udders. The udder of Sophie had gland tissue over practically its entire area in both front and rear quarters (figs. 8 and 10); whereas Blackbird's udder had in the rear quarter an area of gland tissue somewhat irregular and pointed in shape, about 5½ inches at its maximum height and 3 inches at its maximum width, and surrounded laterally and superiorally with solid fat (fig. 9). In the front quarter (fig. 11) the area of gland tissue extended for about 1½ inches along and just beneath the skin and had a maximum depth of about five-eighths inch. The gland tissue was little more than sufficient to inclose the duct or cistern, which was not more than one-fourth inch in diameter, horizontal in position, and approached the teat canal from the rear. The front of this section, which was only approximately three-fourths inch thick, was of solid fat and showed no trace of gland tissue. The relatively great deposition of fat in Blackbird's udder may have been influenced to some extent by her longer dry period. This is of interest, but the significant point about her udder is the extremely small quantity of gland tissue present. This could hardly have been caused by the length of dry period. Udders from dairy cows that had been dry for extended periods showed a distribution of the mammary tissue throughout almost all of the gland. The small quantity of secretory tissue in Blackbird's udder appears, therefore, to have been an inherent characteristic of the cow. It is obvious that the udder of Blackbird was extremely limited in capacity for milk production.

SKELETON

CLEANING AND MOUNTING

The skeleton of each cow has been cleaned and mounted.[3] The cleaning of the bones was accomplished by a chemical process, without boiling, with the result that the texture of the bones, all the finest and most delicate bony structures, and the cartilage were preserved without injury. Except for the head and limbs, each skeleton was prepared, without disarticulation, in four units: (1) The neck; (2) the entire thoracic cage, consisting of vertebrae, ribs, and sternum, which was preserved intact; (3) the lumbar and pelvic portions; and (4) the tail. The hyoid also was preserved. The skeleton of each cow was mounted without significant alteration of shape or position of any bone, according to detailed measurements of the cow made before death. The methods employed have resulted in skeletons

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[3] The skeleton work was done by C. E. Mirguet, an expert osteologist connected with the Smithsonian Institution in Washington, D. C.

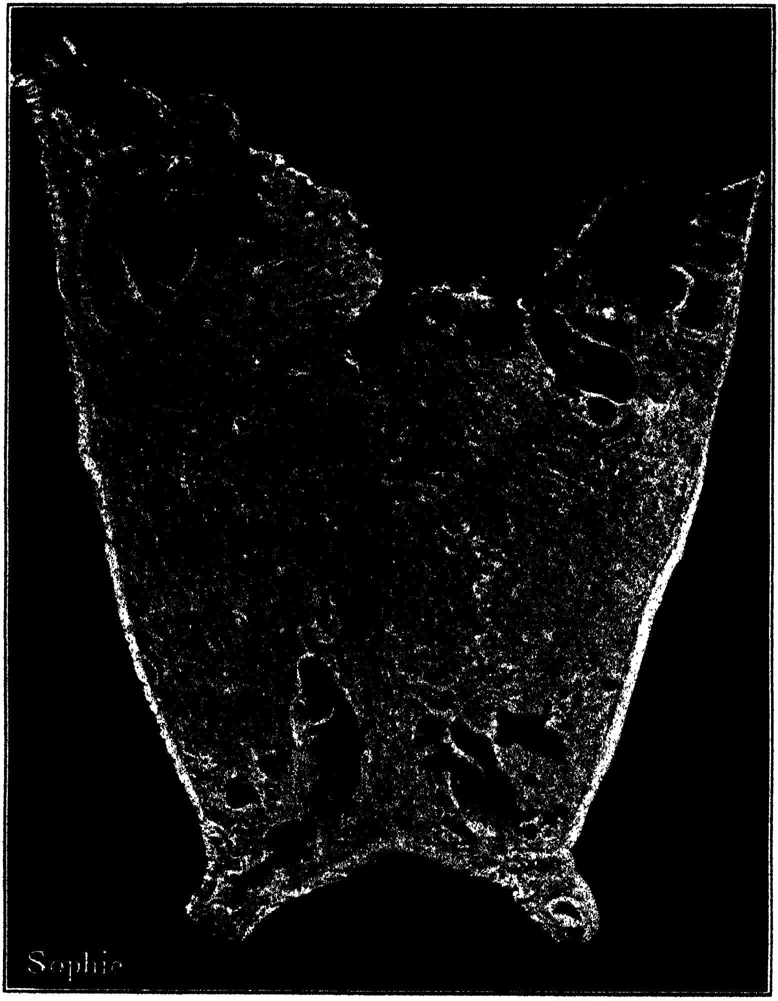

FIG. 8.—Vertical transverse section through the two rear quarters of Sophie's udder

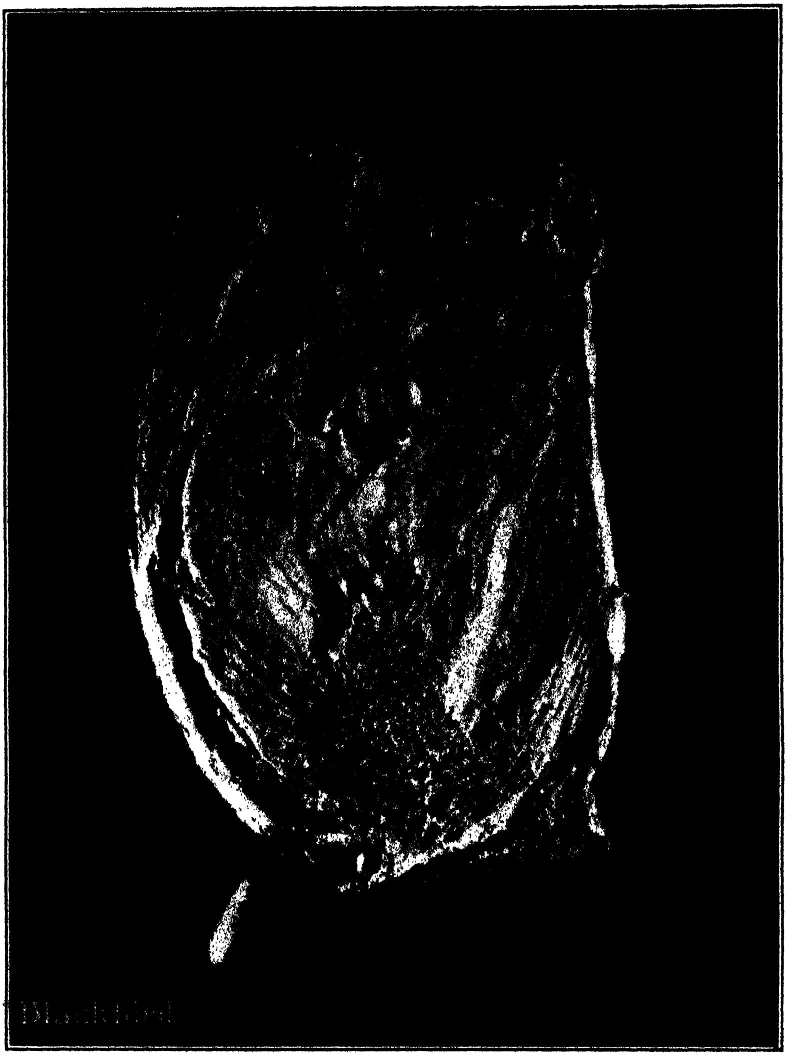

FIG. 9.—Vertical transverse section through one rear quarter of Blackbird's udder

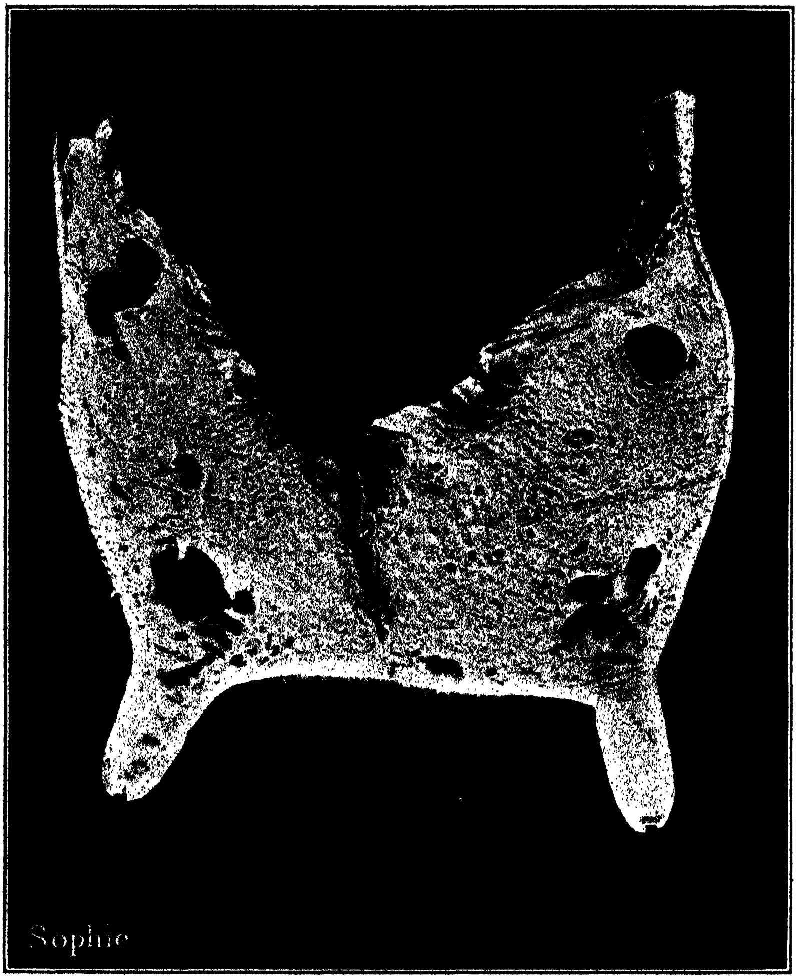

FIG. 10.—Vertical transverse section through the two front quarters of Sophie's udder

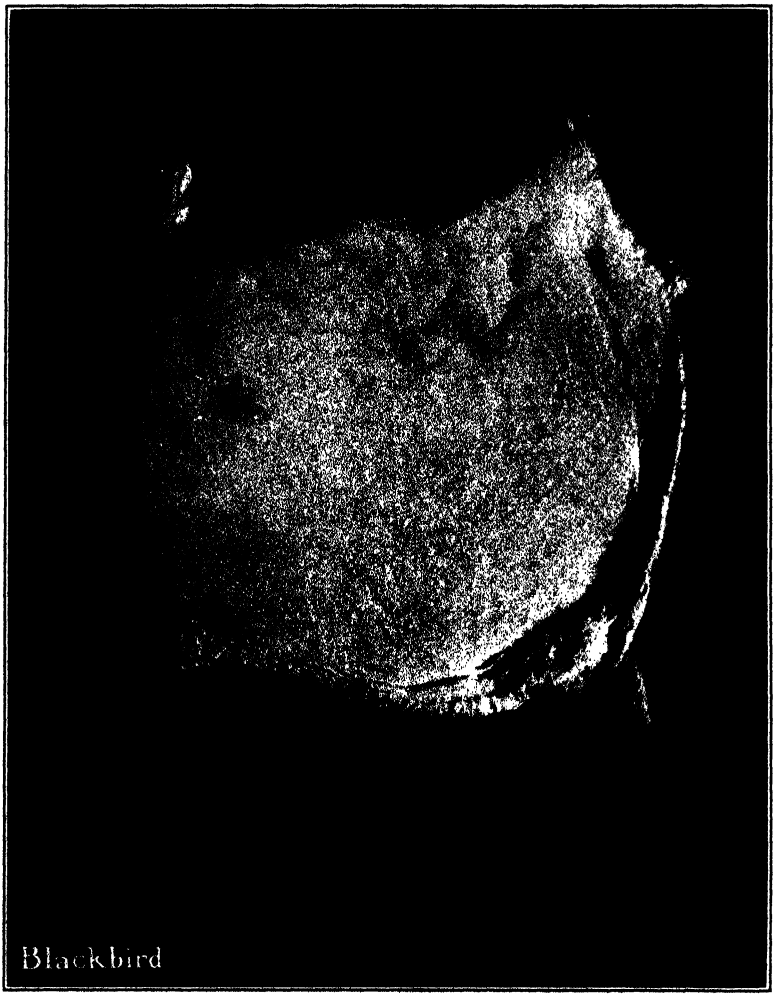

FIG. 11.—Vertical transverse section through one front quarter of Blackbird's udder

which are considered unusually natural in shape and true to life. The two skeletons are illustrated in Figures 12, 13, and 14.

COMPARISONS OF MEASUREMENTS

The two skeletons have been measured in detail. Since the position of the bones of the feet and legs, particularly those of the thoracic limb, are subject to considerable variation, few measurements were made which were based on their position, and relatively little significance can be attached to them except as each bone is considered as a unit.

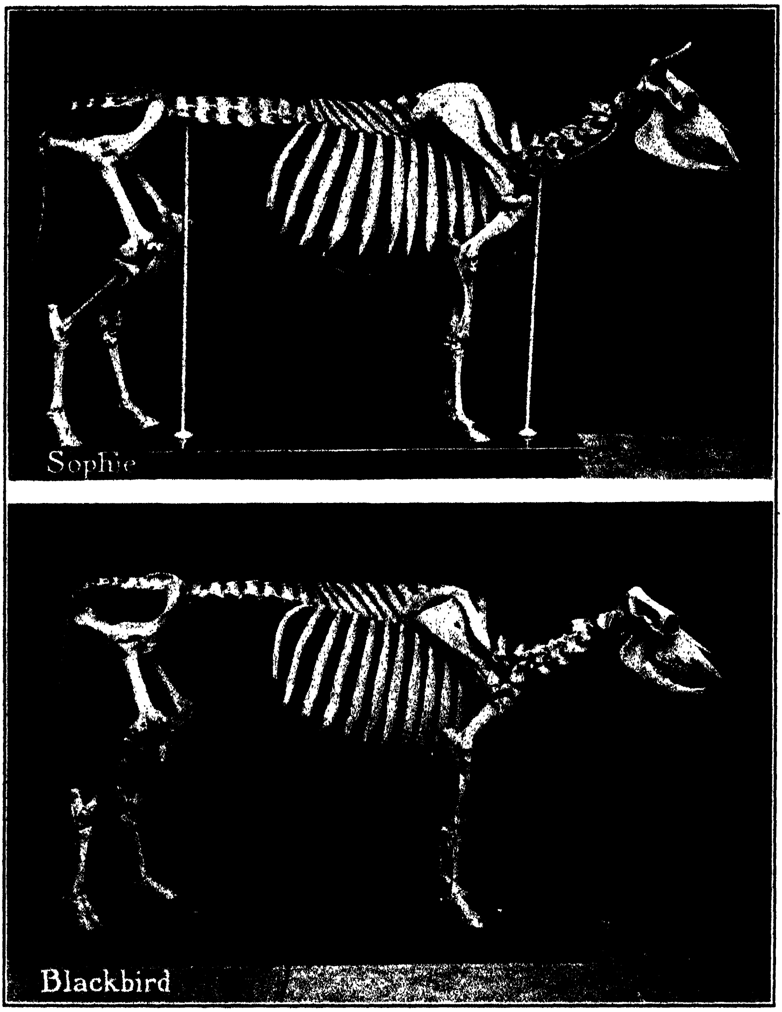

FIG. 12.—Side views of skeletons of Sophie and Blackbird

In order to show graphically the differences in the shape of the bony cage of the two cows, external skeletal contours of the fore

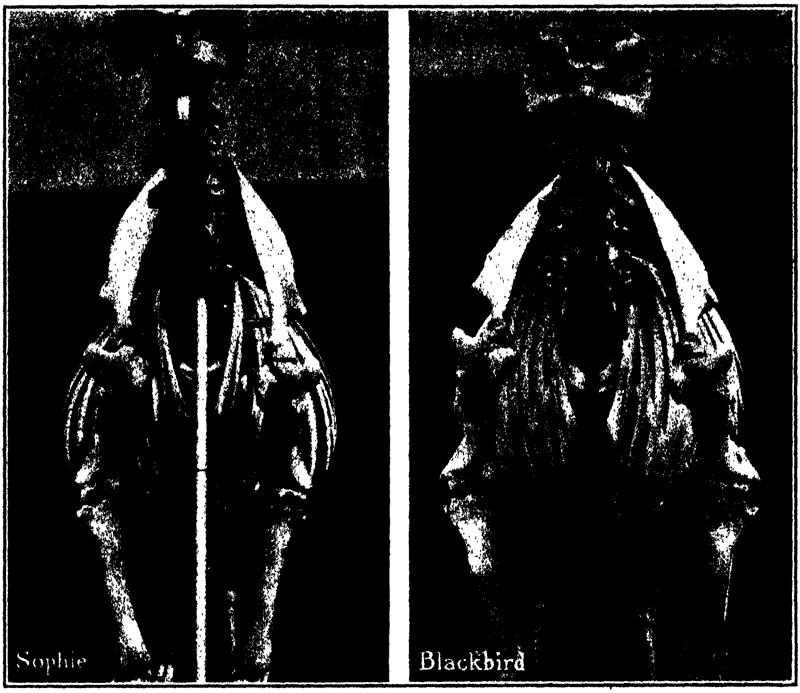

FIG. 13.—Front views of skeletons of Sophie and Blackbird

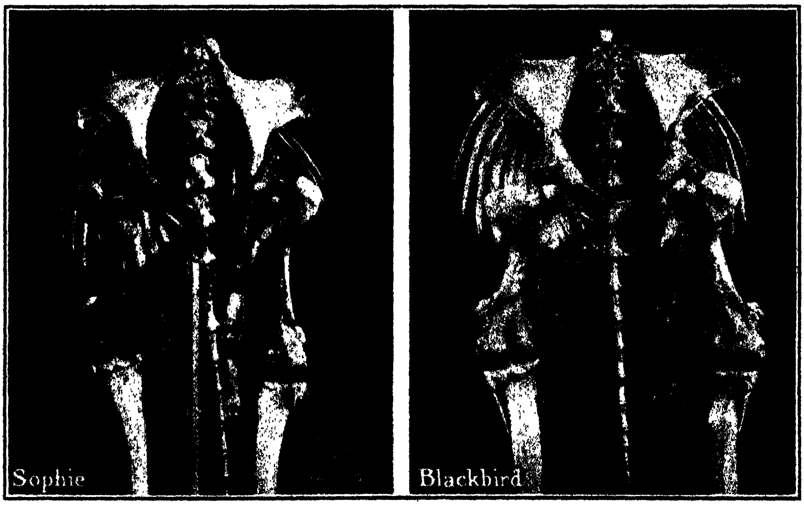

FIG. 14.—Rear views of skeletons of Sophie and Blackbird

chest and paunch have been prepared. Figure 15 shows the fore-chest contours of the two cows, and Figure 16 shows the contours through the paunch. Obviously the paunch contours could not be completed since the extremities of the ribs do not meet.

The fore-chest contours of the two cows are distinctly different in shape. The contour of Sophie is narrower but deeper than that of Blackbird. The areas, however, are almost identical, being 1,439 sq. cm. for Sophie and 1,418 sq. cm. for Blackbird. At the customary plane for taking contours of the paunch, the rear ribs of Blackbird are more highly arched (greater spring of rib), whereas Sophie's ribs are straighter and extend considerably lower. By drawing a straight line connecting the rib extremities for each cow, the following contour areas of paunch were determined: for Sophie, 2,396 sq. cm.; for Blackbird, 2,107 sq. cm. The bony cages of the two cows differ greatly in shape. The cross-section areas at the fore chest are similar, but the cross-section area at the paunch is distinctly smaller for Blackbird than for Sophie. The area for Blackbird is 98.5 per cent of that of Sophie at the fore chest but only 87.9 per cent at the paunch.

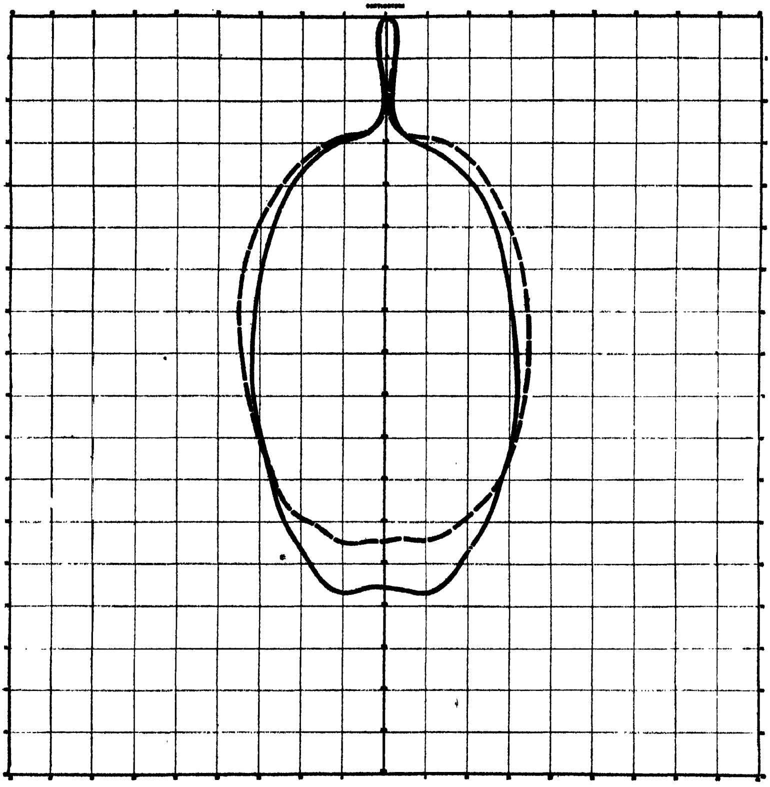

FIG. 15.—External contours of fore chest of skeleton. Solid line represents the contour of Sophie; broken line that of Blackbird

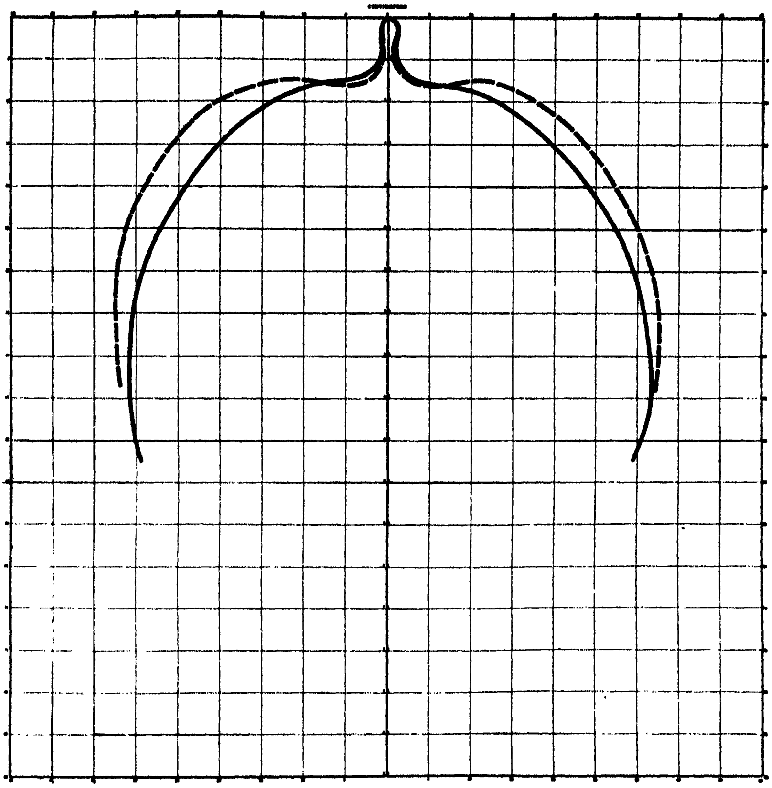

FIG. 16.—External contours of paunch of skeleton. Solid line represents the contour of Sophie; broken line that of Blackbird

The actual skeletal measurements of Sophie and Blackbird and their relative values, expressed in percentage of Sophie's measurements, are shown in Table 5.

TABLE 5.—*Detailed skeletal measurements of Sophie and Blackbird*

Item	Sophie	Blackbird	Relation of Blackbird's data to those of Sophie
	Cm.	*Cm.*	*Per cent*
Height at top of highest thoracic spinous process	120.00	113.75	94.8
Height at highest point on sacrum	121.25	117.75	97.1
Height at top of hip points	115.75	113.63	98.2
Height at top of pin bones	106.25	105.50	99.3
Length between bearing surfaces:			
Bones of thoracic limb—			
Scapula	37.00	34.00	91.9
Humerus	27.00	25.00	92.6
Radius	28.00	26.00	92.9
Metacarpus	20.50	19.00	92.7
Total	112.50	104.00	
Average	28.13	26.00	92.4

TABLE 5.—*Detailed skeletal measurements of Sophie and Blackbird*—Continued

Item	Sophie	Blackbird	Relation of Blackbird's data to those of Sophie
Length between bearing surfaces:			
Bones of pelvic limb—	*Cm.*	*Cm.*	*Per cent*
Femur	35.50	34.00	95.8
Tibia	33.50	31.00	92.5
Metatarsus	23.00	23.00	100.0
Total	92.00	88.00	----------
Average	30.67	29.33	95.7
Height at poll	129.00	111.50	86.4
Length, poll to third thoracic process	71.00	64.00	90.1
Length, third thoracic process to point on spine between hips	89.50	89.00	99.4
Length, point on spine between hips to rear of pin bones	47.00	41.00	87.2
Length, third thoracic process to rear of pin bones	136.50	130.00	95.2
Total length, poll to rear of pin bones	207.50	194.00	93.5
Sway of back	5.00	2.50	50.0
Width of fore chest (outside, sixth rib)	33.25	33.75	101.5
Width of rear chest (outside, crossing center tenth rib)	55.00	52.50	95.5
Width of paunch (outside, crossing center thirteenth rib)	62.50	62.50	100.0
Depth of fore chest (plane of sixth rib)	68.50	62.25	90.9
Maximum lateral width of thoracic cavity at anterior edge of each rib:			
1	9.50	9.50	100.0
2	11.50	11.00	95.7
3	15.50	15.00	96.8
4	19.50	20.50	105.1
5	23.50	25.50	108.5
6	27.50	29.50	107.3
7	33.00	34.00	103.0
8	41.50	41.00	98.8
9	49.00	46.00	93.9
10	55.00	50.50	91.8
11	60.00	56.00	93.3
12	62.00	60.50	97.6
13	62.50	63.00	100.8
Average	36.15	35.54	98.3
Vertical depth from ventral center of each thoracic vertebra to sternum or to a horizontal line across the lower extremity of the thoracic cage at the eighth to thirteenth vertebrae:			
1	26.00	23.00	88.5
2	31.00	27.00	87.1
3	34.00	30.00	88.2
4	38.00	33.50	88.2
5	41.50	35.50	85.5
6	44.50	38.00	85.4
7	49.00	40.50	82.7
8	47.00	42.00	89.4
9	45.00	42.50	94.4
10	44.50	43.00	96.6
11	45.00	42.50	94.4
12	43.00	41.00	95.3
13	41.00	39.50	96.3
Average	40.73	36.77	90.3
Length, center of anterior edge of first rib to junction of thoracic and lumbar vertebrae (length of thoracic cavity)	76.00	72.50	95.4
Center axes, anterior aperture of thoracic cage:			
Lateral	8.00	8.00	100.0
Vertical	18.50	18.00	97.3
Contour or cross-section area at anterior aperture of thoracic cage	172.90	151.50	87.6
Contour area of thoracic cage (inside) at seventh rib	1,363.60	1,268.50	93.0
Contour area of thoracic cage (inside) at thirteenth rib	2,135.70	1,985.50	93.0
Height at anterior dorsal point of sternum (ventral point of anterior aperture of thoracic cage)	72.00	64.00	88.9
Height at dorsal median posterior point on sternum between attachment of eighth ribs	52.50	53.50	101.9
Length of sternum between above points	40.00	34.50	86.3
Angle of sternum made with the horizontal	([a])	([a])	60.7
Length of thoracic cage measured on spine (cervical-thoracic to thoracic-lumbar junctions)	76.00	71.00	93.4
Average length each thoracic vertebra (divide above length by 13)	5.85	5.46	93.3
Length of loin measured on spine (thoracic-lumbar to lumbar-sacral junctions)	41.00	37.50	91.5
Average length each lumbar vertebra (divide above length by 6)	6.83	6.25	91.5

[a] Sophie, 29° 11′; Blackbird, 17° 43′

TABLE 5.—*Detailed skeletal measurements of Sophie and Blackbird*—Continued

Item	Sophie	Blackbird	Relation of Blackbird's data to those of Sophie
Length of spinous process of each thoracic vertebra: [b]	*Cm.*	*Cm.*	*Per cent.*
1	25.00	22.00	88.0
2	25.00	22.00	88.0
3	24.50	22.00	89.8
4	23.50	21.00	89.4
5	21.50	19.50	90.7
6	20.50	19.00	92.7
7	20.00	18.00	90.0
8	18.50	17.00	91.9
9	17.00	16.00	94.1
10	14.50	14.00	96.6
11	12.00	12.00	100.0
12	9.00	9.00	100.0
13	7.50	7.50	100.0
Average	18.35	16.85	91.8
Length of spinous process of each lumbar vertebra:			
1	6.50	6.50	100.0
2	6.00	6.00	100.0
3	5.50	6.00	109.1
4	5.50	5.00	90.9
5	5.00	4.50	90.0
6	4.50	4.50	100.0
Average	5.50	5.42	98.5
Width of each rib, 6 inches from its lowest ossified point (average of right and left):			
1	2.13	2.32	108.9
2	2.42	2.31	95.5
3	3.31	2.92	88.2
4	4.42	3.88	87.8
5	5.05	4.35	86.1
6	5.23	4.47	85.5
7	5.81	4.93	84.9
8	5.41	4.54	83.9
9	4.92	4.15	84.3
10	4.69	3.66	78.0
11	3.96	3.45	87.1
12	3.19	2.48	77.7
13	3.29	2.20	66.9
Total	53.83	45.66	----------
Average	4.14	3.51	84.8
Length of each rib, from vertebral attachment to lowest ossified point (average of right and left):			
1	27.00	26.50	98.1
2	32.00	30.75	96.1
3	36.00	35.50	98.6
4	39.25	39.25	100.0
5	43.75	44.50	101.7
6	48.00	48.00	100.0
7	52.50	52.00	99.0
8	55.25	55.25	100.0
9	55.25	54.25	98.2
10	55.25	54.00	97.7
11	54.00	53.00	98.1
12	52.25	51.50	98.6
13	48.50	46.50	95.9
Average	46.08	45.46	98.7
Width of intercostal spaces (spaces between ribs) approximately 6 inches from lowest ossified point:			
1	1.89	1.97	104.2
2	2.15	2.55	118.6
3	1.96	2.41	123.0
4	1.80	1.97	109.4
5	1.80	1.71	95.0
6	2.76	2.53	91.7
7	1.30	2.00	153.8
8	3.75	3.05	81.3
9	4.51	3.58	79.4
10	4.78	4.37	91.4
11	4.92	5.03	102.2
12	5.07	5.18	102.2
Total	36.69	36.35	----------
Average	3.06	3.03	99.1

[b] Those of Sophie were set at extreme angle. Those of Blackbird were much more nearly vertical.

TABLE 5.—*Detailed skeletal measurements of Sophie and Blackbird*—Continued

Item	Sophie	Blackbird	Relation of Blackbird's data to those of Sophie
Diameter of foramina of thoracic vertebrae (intervertebral and intravertebral):	*Cm.*	*Cm.*	*Per cent*
1	1.22	1.25	102.5
2	1.16	.57	49.1
3	1.21	.48	39.7
4	1.05	.61	58.1
5	.94	.83	88.3
6	.92	.55	59.8
7	.94	.70	74.5
8	.87	.71	81.6
9	.98	.80	81.6
10	1.10	.76	69.1
11	.84	.68	81.0
12	.74	.64	86.5
13	2.94	.78	26.5
Average	1.15	.72	62.8
Diameter of foramina of lumbar vertebrae (intervertebral and intravertebral):			
1	2.20	1.28	58.2
2[c]	**2.55**	**1.68**	**65.9**
3	2.79	1.91	68.5
4	2.83	2.44	86.2
5	2.86	2.32	81.1
6	2.24	1.97	87.9
Average	2.58	1.93	75.0
Length of loin, from center of hip to vertebral attachment of thirteenth rib	45.00	42.00	93.3
Width of loin:			
Third lumbar	29.00	29.75	102.6
Fourth lumbar	30.25	32.50	107.4
Average	29.63	31.13	105.1
Width of hips (outside extremity)	46.50	47.25	101.6
Width of thurls (outside extremity)	40.50	39.25	96.9
Width of pin bones (outside extremity)	27.50	23.75	86.4
Length of rump, top of hip to top of pin bone	40.00	35.00	87.5
Angle of rump	([d])	([d])	97.8
Angle of floor of pelvis	([e])	([e])	124.5
Angle of pelvic aperture	([f])	([f])	106.4
Length of pelvic floor	17.50	17.00	97.1
Center axes of pelvic aperture:			
Lateral	25.00	24.50	98.0
Longitudinal	17.20	15.50	90.1
Contour area of pelvic aperture	392.00	350.90	89.5
Width between top points of pin bones	20.00	17.00	85.0
Width of pelvis immediately above center of acetabulum	17.75	16.50	93.0
Width of forehead	14.25	15.75	110.5
Width across eyes	20.75	22.25	107.2
Length from poll to tip of nose	47.00	45.50	96.8
Greatest depth at angle of jaw	26.00	24.25	93.3
Measurements of muzzle:			
Outside width of premaxillae (nasal processes)—			
At narrowest point corresponding to corner of mouth	7.66	7.64	99.7
Near anterior extremity, corresponding to greatest width of prehensile pad	8.28	8.50	102.7
Outside width of mandible (lower jaw)—			
At narrowest point, corresponding to corner of mouth	3.50	4.05	115.7
Near anterior extremity, corresponding to lateral limits of incisor teeth	7.74	7.45	96.3
Inside diameters of nasal passage—			
Lateral diameter	6.72	6.42	95.5
Depth from roof to floor	6.05	5.65	93.4
Measurements of bones of thoracic limb (scapula not included). Center of shaft of:			
Humerus—			
Circumference	14.00	15.00	107.1
Lateral diameter	3.92	4.32	110.2
Anterior-posterior diameter	4.87	5.17	106.2
Radius—			
Circumference	13.00	14.50	111.5
Lateral diameter	4.64	5.47	117.9
Anterior-posterior diameter	3.17	3.50	110.4

[c] Opening for nerve branch that goes to udder.
[d] Sophie 13° 44′; Blackbird 13° 26′.
[e] Sophie 16° 36′; Blackbird 20° 40′.
[f] Sophie 38° 19′; Blackbird 40° 46′.

TABLE 5.—*Detailed skeletal measurements of Sophie and Blackbird*—Continued

Item	Sophie	Blackbird	Relation of Blackbird's data to those of Sophie
Measurements of bones of thoracic limb (scapula not included)—Continued.			
Center of shaft of:—Continued.			
Metacarpus—	*Cm.*	*Cm.*	*Per cent*
Circumference	9.50	10.75	113.2
Lateral diameter	3.30	3.87	117.3
Anterior-posterior diameter	2.29	2.54	110.9
Average	6.52	7.24	111.0
Measurements of bones of pelvic limb. Center of shaft of:			
Femur—			
Circumference	12.50	13.50	108.0
Lateral diameter	3.53	3.85	109.1
Anterior-posterior diameter	4.30	4.64	107.9
Tibia—			
Circumference	11.50	13.00	113.0
Lateral diameter	4.10	4.96	121.0
Anterior-posterior diameter	2.93	2.86	97.6
Metatarsus—			
Circumference	9.50	11.00	115.8
Lateral diameter	2.78	3.20	115.1
Anterior-posterior diameter	2.90	3.53	121.7
Average	6.00	6.73	112.0

The skeleton of Sophie is slightly taller and about 6 or 7 per cent longer than that of Blackbird. The head and front quarters of Blackbird are relatively low. The total length of the bones of the front leg of Blackbird is only 92.4 per cent of that of Sophie, whereas the length of the bones of the hind leg is 95.7 per cent as great. Sophie has a greater dip or sway of back than Blackbird.

Blackbird is slightly greater than Sophie in external width of fore chest, narrower in rear chest, the same in width of paunch, but decidedly less in depth of fore chest. Measurements of the internal widths of thoracic cavity taken at the anterior edge of each rib show on an average nearly the same values for each cow, although Blackbird is very slightly wider than Sophie in the front half and very slightly narrower in the rear half of the thorax. The internal vertical depths from the center of each vertebra to the sternum show that Blackbird is consistently shallower, averaging only 90.3 per cent the depth of Sophie. The length of thoracic cavity also is slightly less for Blackbird than for Sophie. The anterior aperture of the thoracic cavity is of nearly the same dimensions for each cow, but the contour areas of the openings are decidedly less for Blackbird than for Sophie. The areas of the inside contours across the seventh and thirteenth ribs have the same relationship for the two cows, being in each case 93 per cent as great for Blackbird as for Sophie. Blackbird's sternum is lower in front, very slightly higher at the rear and much shorter. It has, therefore, a very much smaller angle of inclination from the horizontal, indicating that in skeletal structure she had less vertical wedge shape than Sophie. On the contrary, the widths of fore chest, rear chest, and paunch indicate that in skeletal structure the two cows exhibited almost exactly the same degree of wedge shape laterally. Heavy fleshing in the region of the shoulders and fore chest undoubtedly was responsible for the fact that Blackbird when living showed relatively very little of the lateral wedge shape externally. Blackbird's thoracic cavity is shorter, and the average length of her thoracic vertebrae is correspondingly less than that of Sophie. Similarly, the lengths of the lumbar region and of each lumbar vertebra of Blackbird are considerably shorter than those of Sophie.

A marked difference exists between the spinous processes of the thoracic vertebrae of the two cows. Those of Sophie incline backward at a distinct angle, whereas those of Blackbird are much more nearly vertical. The angle of inclination of these processes was not measured, but the differences are clearly indicated in Figures 17 and 18. In length, the processes of Sophie are considerably greater than those of Blackbird. This difference is distinctly noticeable in the anterior thoracic vertebrae but diminishes steadily as the more posterior vertebrae are approached, the eleventh, twelfth, and thirteenth being of equal length in both cows. On an average the processes of Blackbird are 91.8 per cent as long as those of Sophie. The lumbar processes of both cows are similar in shape and in length.

One outstanding difference between the two cows is the width of ribs measured 6 inches above the lowest ossified point. Except for the first rib those of Sophie are in every case wider than those of Blackbird. The difference increases rather steadily from the second to the thirteenth rib. On an average those of Blackbird are 84.8 per cent as wide as those of Sophie.

The length of ribs is approximately the same for each cow, although Blackbird's are more arched in the upper or dorsal portion. The width of spaces between ribs varies considerably at certain points, but on an average they are almost identical. The greater width of each rib of Sophie with equal intercostal spaces gives her a considerably greater length of thoracic cavity in the region of the lower part of the rib.

Judges of dairy cattle are inclined to attach considerable importance to the openness of conformation or the width of the spaces between ribs, spinous processes, and vertebrae, believing that such openness allows more space for the nerves to pass out through the spine from the spinal cord. According to Sisson[4] "The notches of two adjacent vertebrae form intervertebral foramina (*Foramina intervertebralia*) for the passage of the spinal nerves and vessels; in some vertebrae, however, there are complete foramina instead of notches." Attention should be called to the fact that there are more intravertebral than intervertebral foramina in these skeletons. The intravertebral foramina appear near the center of the vertebrae and could hardly be associated with the width of spaces between the tops of the spinous processes. Furthermore, the intervertebral foramina are nearly all in the region of the lumbar vertebrae where the distances between the tops of the processes are not readily determined by examining the living animal. It appears, therefore, that judgment of the width of spaces between spinous processes in the living animal is not particularly significant of the freedom of passage of nerves from the spinal cord. These foramina, whether intervertebral or intravertebral, have been measured as accurately as possible.

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[4] SISSON, S. THE ANATOMY OF THE DOMESTIC ANIMALS. Ed. 2, p. 26. Philadelphia and London, W. B. Saunders Co. 1914.

Because of their location and position, measurement of some of them was difficult. Most of them are nearly round, but some are irregular in shape. The measurements recorded are the greatest transverse diameters of the openings. In a few cases the vertebrae show both intervertebral and intravertebral foramina. In a few instances, especially in the lumbar region, the foramina are divided either by cartilage or bone into two parts. Wherever a vertebra shows two foramina or one divided into two parts, both have been measured and the total of the two diameters recorded for that vertebra. The foramina of Blackbird are very much smaller than those of Sophie. On an average the thoracic foramina of Blackbird are 62.8 per cent as large as those of Sophie. The corresponding relative size for lumbar foramina is 75 per cent. The lumbar and thoracic vertebrae of Sophie and Blackbird are shown in Figures 17 and 18.

Smith [5] states:

> The mammary gland is innervated in quadrupeds (in addition to the ileo-inguinal nerve distributed to the skin) by the external spermatic nerve. This nerve originates from the lumbar portion of the spinal cord and passes out between the greater and lesser psoas muscles, dividing in the pelvis into three branches, of which one is distributed to the abdominal muscles, while the other two leave the abdominal cavity through the femoral ring accompanying the crural artery, and then, following the course of the external pudic artery, are distributed to the mammary gland.

Sisson [6] specifically describes the origin of this nerve as follows:

> It (one of the branches of the second lumbar nerve) joins a branch of the external spermatic nerve, and the trunk so formed descends the inguinal canal, to be distributed to the external genital organs and the surrounding skin in the inguinal region. * * * The external spermatic nerve (*N. spermaticus externus*, third lumbar nerve) passes backward in the substance of the psoas minor and divides into two branches.[7]

One of these branches emerges behind the circumflex iliac vessels and—

> runs lateral to and parallel with the external iliac artery and descends in the medial part of the inguinal canal. It emerges at the external ring with the external pudic artery and ramifies in the external genital organs and the skin of the inguinal region. * * * The origin and disposition of some of the foregoing nerves are variable. In some cases the ilio-inguinal nerve ends in the psoas major, and appears then to be absent. The mode of formation of the inguinal nerves is inconstant.

It appears that the nerves which control the udder leave the spinal cord and pass through the foramina between the second and third lumbar vertebrae. The diameters of these foramina are 2.55 cm. for Sophie and 1.68 cm. for Blackbird, as shown in Table 5. The foramina of Blackbird, therefore, have diameters only 65.9 per cent as great as those of Sophie. The significance of these diameters, however, may be subject to speculation.

Blackbird has a shorter but slightly wider loin than Sophie, and her lateral processes turn upward instead of downward at the ends as in the case of Sophie. The width of hips is very slightly greater, whereas width of thurls, width of pin bones, and length of rump are less for Blackbird than for Sophie.

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[5] SMITH, R. M. THE PHYSIOLOGY OF THE DOMESTIC ANIMALS. p. 629. Philadelphia and London, F. A. Davis. 1889.

[6] SISSON, S. Op. cit., pp. 822–823.

[7] According to the diagram on p. 822 of Sisson the external spermatic nerve appears to emerge between the second and third lumbar vertebrae.

In angle of rump the two skeletons are similar. This may appear to be inconsistent, since the angle of rump on the living animal was somewhat less for Blackbird than for Sophie. Part of this discrepancy is due to the difficulty experienced in measuring the body

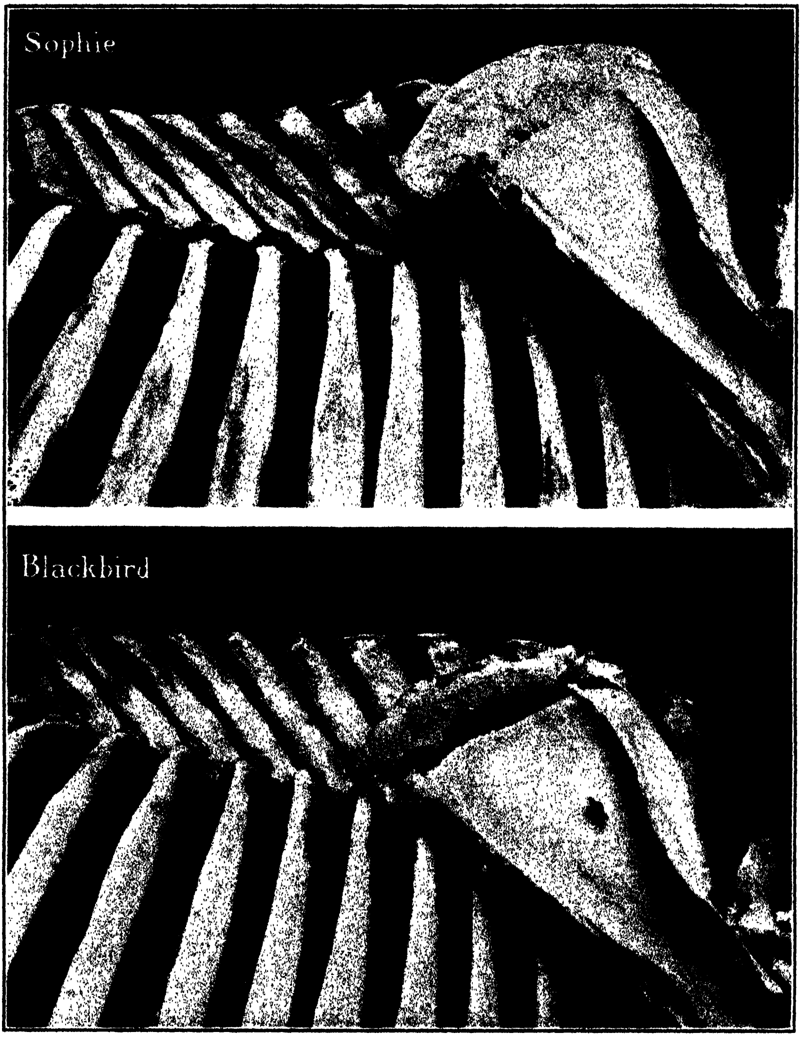

Fig. 17.—Vertebrae, ribs, and spinous processes of thorax of Sophie and Blackbird showing foramina or openings through the vertebrae for the passage of nerves and vessels

of Blackbird because of the amount of subcutaneous fat over her hips and pin bones. The skeleton also shrinks in drying, which results in a tendency for the spine to become slightly arched. A very slight difference in the amount of arching may affect the angle of inclination of the rump or pelvis. This was carefully guarded against but may not have been entirely avoided. The shrinkage of the skeleton also accounts for some of the reductions in skeletal lengths as compared with similar measurements of the living form.

The floor of the pelvis tends to incline upward toward the rear. The angle is considerably greater for Blackbird than for Sophie. The angle which the plane of the pelvic aperture, or anterior opening of the pelvis, makes with a horizontal is not greatly different for the two cows. The length of pelvic floor is approximately the same for both cows. The axis of the pelvic aperture of Blackbird is about the same laterally as that of Sophie but is considerably less longitudinally. The contour or cross-section area of the pelvic aperture is distinctly greater for Sophie than for Blackbird. In width of pelvis both across the top points of the pin bones and across the center of the acetabulum, the measurements are greater for Sophie than for Blackbird. It appears that Sophie had a greater space for

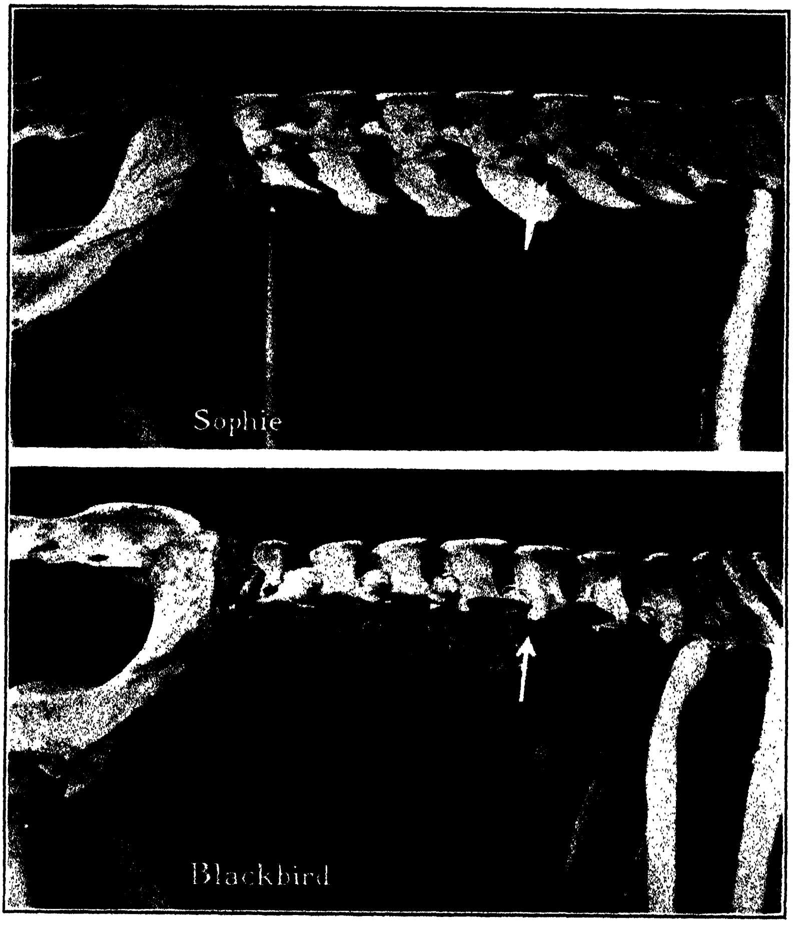

FIG. 18.—Lumbar vertebrae and spinous processes of Sophie and Blackbird showing intravertebral and intervertebral foramina. Arrows indicate foramina which permit passage of nerve which innervates the mammary gland

delivery of fetus than did Blackbird. In this connection it is of interest to note the birth weight of Jersey and Aberdeen Angus calves. The average birth weight of 83 Jersey calves recorded at the Beltsville, Md., station is 57.3 pounds. Assembled data, furnished by four experiment stations,[8] indicate an average birth weight of 69 pounds for 424 Aberdeen Angus calves.

Blackbird is wider in the forehead and across the eyes, whereas Sophie is longer from poll to tip of nose. Sophie also is deeper through the maximum depth of jaw. The widths of nasal processes are similar for the two cows. The widths of mandible are nearly the same, that of Sophie being slightly greater at one point of measurement and slightly less at the other. The inside diameters of the nasal passage, however, are both slightly greater for Sophie than for Blackbird. The muzzle measurements of the skeletons are strikingly similar. The circumference of muzzle of the two cows when living was 108.5 per cent as great for Blackbird as for Sophie.

The circumference, the lateral diameter, and the anterior-posterior diameter of the humerus, radius, metacarpus, femur, tibia, and metatarsus were measured. In all except 1 of the 18 measurements, the values are greater for Blackbird than for Sophie. The one exception is the anterior-posterior diameter of the tibia which, because of its shape, was particularly difficult to measure. The leg bones of Blackbird are distinctly heavier or greater in diameter than those of Sophie, not only in proportion to their length, but also in actual units of measurement.

SUMMARY

EXTERNAL FORM OF LIVING ANIMAL

Blackbird was in very high condition of flesh and had a deposition of subcutaneous fat as great as 9 cm. in thickness in some places. Sophie had very little body fat and weighed 638 pounds less than Blackbird.

Blackbird was slightly higher at the pin bones, of approximately the same height at the hips, but lower at the withers. On an average she was 98.8 per cent as tall as Sophie. She also was shorter in body than Sophie.

Blackbird was distinctly deeper in body and showed a far greater vertical wedge shape than Sophie. She was much wider but exhibited only about half as much lateral wedge shape as did Sophie. Body circumferences and contour or cross-section areas of fore chest and paunch were much greater for Blackbird than for Sophie, yet both measurements indicated greater body wedge shape for Sophie.

Blackbird's body surface area appeared to be about 20 per cent greater than that of Sophie.

The calculated volume of barrel was nearly 70 per cent greater for Blackbird than for Sophie.

Blackbird was wider in proportion to depth both at the fore chest and paunch, with resulting lower thoracic and abdominal indexes than those of Sophie.

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[8] Acknowledgment is made for the assistance rendered by members of the animal husbandry departments of the Illinois, Mississippi, Nebraska, and Ohio experiment stations in furnishing these data.

Blackbird was slightly more nearly level in the rump than was Sophie. The influence of fat deposition on this point is speculative.

The legginess of Blackbird was approximately 87 per cent of that of Sophie.

Heaviness of bone, as judged by circumference of shin bone or metacarpus of the living animal, was considerably greater for Blackbird than for Sophie.

Blackbird had a slightly shorter head, wider forehead, and greater circumference of muzzle.

The greater height, greater length of body, and greater length of head are all indicative of greater " scale " for Sophie.

Only five of the measurements that are greater for Blackbird than for Sophie are more than 25 per cent in excess. In each of these five items, the marked difference between the two cows can be attributed largely to the heavy fleshing and fat deposition of Blackbird.

INTERNAL ANATOMY

The empty body weight of Blackbird was 707.8 pounds, or 92.1 per cent greater than that of Sophie. As might be anticipated under these conditions, the weights of organs or body parts per 100 pounds of empty body weight were much lower for Blackbird than for Sophie.

The weights of kidneys and of adrenals were distinctly greater for Blackbird than for Sophie.

The weight of spleen was almost the same for both cows.

The weights of empty stomachs and of empty intestines were much less for Blackbird than for Sophie.

Blackbird had 181.71 feet of intestines as compared with 166.50 feet for Sophie. The intestinal lengths of Sophie and Blackbird are about intermediate to the range of intestine lengths obtained from a large group of cows.

An accurate weight of Sophie's lungs was not obtained. Blackbird's lungs appeared to be relatively low in actual weight and in weight per 100 pounds empty body weight when compared with similar data obtained from a large group of cows.

The weight and circumferences of heart were similar for both cows. Both were below the average heart weight obtained on a large number of cows. Furthermore, the weight of heart per 100 pounds of empty body weight was relatively low for Blackbird.

The weight of thyroid was very much greater for Blackbird than for Sophie. This can be attributed largely to the interlying of fat in the case of Blackbird.

Except for the weight of intestines, intestine contents, stomachs, stomach contents, heart, and one of the heart circumferences, all the weights and measurements given in Table 4 are greater for Blackbird than for Sophie. The difference in weight of heart is so small that it is negligible.

MAMMARY GLAND

Externally, the udder of Blackbird, as compared with that of Sophie, was more compact and more closely attached with less looseness, greater width, and less depth. It appeared to have a greater total quantity of tissue which was more yielding and much less coarsely and harshly fibrous than that of Sophie. Blackbird had relatively very inferior veins on udder and on abdomen and smaller and less distinct milk wells. The skin covering the udder of Blackbird was thicker, mellower, and less flexible than that of Sophie.

Internally, Sophie's udder had gland tissue over almost the entire area of each of the transverse sections into which it was cut. The rear quarter of Blackbird's udder had only a very small quantity of gland tissue surrounded by a heavy deposition of firm fat. Sections through the front quarter of Blackbird's udder showed little more than a duct or cistern, about a quarter of an inch in diameter and extending longitudinally to the front teat.

SKELETON

Blackbird is not so tall and is shorter in body than Sophie. Her leg bones are shorter and her head and front quarters are lower.

Measurements of the outside of the thoracic cage of the skeletons indicate that Blackbird is slightly wider in fore chest, narrower in rear chest, and of the same width of paunch as Sophie. She also is decidedly shallower in fore chest. The rear ribs of Blackbird are much more highly sprung or arched than those of Sophie.

The internal skeletal measurements of the thoracic cage show on an average almost the same widths for both cows. Blackbird, however, has decidedly less depth and somewhat less length of thorax. The inside contours at the seventh and at the thirteenth ribs are in each case 93 per cent as great for Blackbird as for Sophie. The anterior aperture of the thoracic cage is similar in dimensions for both cows but very much less in area for Blackbird than for Sophie.

Blackbird's sternum is much shorter, decidedly lower in front, and very slightly higher in the rear, giving her decidedly less of the vertical wedge shape. In lateral wedge shape, however, the two skeletons are almost identical, although in their living form, because of difference in fleshing, Sophie had almost twice as much lateral wedge shape as Blackbird.

Blackbird's thoracic vertebrae are shorter than Sophie's, and the spinous processes are shorter and much more nearly vertical. Her ribs are very much narrower but of about the same length as Sophie's, and the width between ribs averages almost the same for both cows.

Blackbird is shorter but wider in the loin and has shorter lumbar vertebrae. The lumbar spinous processes of both cows are approximately the same in shape and in length. The transverse lumbar processes of Blackbird incline upward, whereas those of Sophie are turned downward.

The diameters of thoracic foramina are only 62.8 per cent as great for Blackbird as for Sophie. The corresponding diameters of lumbar foramina are 75 per cent as great for Blackbird as for Sophie. The foramina which allow passage of the nerves which innervate the udder are 65.9 per cent as great in diameter for Blackbird as for Sophie.

Blackbird is very slightly wider at the hips but narrower at the thurls and pin bones. She is shorter in the rump with almost the same angle of rump as Sophie.

Blackbird's pelvis is narrower and slightly shorter on the floor. Both the pelvic floor and the plane of the pelvic aperture have greater angles of inclination than those of Sophie. The pelvic aperture, or anterior opening of the pelvis, is slightly narrower, distinctly shorter, and has a decidedly smaller area, allowing less space for delivery of the fetus, although average birth weights of Jersey and Aberdeen Angus calves are shown to be 57.3 and 69 pounds respectively.

Blackbird has a wider but slightly shorter head and a shallower jaw. The muzzles of the two cows are similar in width. Blackbird has a somewhat smaller nasal passage.

The leg bones of Blackbird are distinctly heavier, and greater in lateral and anterior-posterior diameter, both in actual measurements and in proportion to their length.

Obviously a comparison of large numbers of dairy and beef skeletons is at present impossible because of the limited material available. The foregoing data are presented to show the general differences between these two skeletons. It is not to be inferred that the same or similar differences would be found between all dairy and beef skeletons.

GENERAL

In external form the two cows differed greatly.

In weight and size of internal organs, the differences were not sufficiently great to indicate significant differences in function.

In skeleton structure the two cows varied somewhat but were generally similar. This would indicate that the evolution of the dairy and beef types, which has been accomplished through breeding and selection, has not materially altered their skeletal structure, but rather that the difference in type is due to extreme fleshing on the one hand and to udder development and absence of fleshing on the other.

Aside from the external form, the most marked difference noted between the cows compared was the quantity of secretory tissue in their udders.

THE USEFULNESS OF CAPILLARY POTENTIAL TO SOIL-MOISTURE AND PLANT INVESTIGATORS[1]

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INTRODUCTION

Soils literature contains numerous discussions of the problem of the movement of moisture in the soil and its availability to the plant. In a great many cases, however, the point of view is that of the experimentalist, and the possible advantages that may accrue from a consideration of the generalizations available in the literature of physics and chemistry are almost wholly overlooked.

One investigator, for example, discovers from his experiments that water evaporates more rapidly from a moist than from a dry soil; another discovers that the moisture tends to accumulate toward the rim of a sample in the centrifugal machine; still another finds that a wet clay will absorb moisture from a sand comparatively drier. Many examples of this character may be pointed out as results of experimentation purporting to represent contributions to scientific knowledge. By such experimental procedure and by careful analysis of the resulting data we may succeed anew in working out generalizations from which we may predict with some degree of certainty, qualitatively at least, the way in which soil water will move under certain conditions. If we are to profit by experience we must make these generalizations, but it is quite probable that in many cases such generalizations will be found to conform with those that are already known to the basic sciences.

It is at once obvious that a mixture of mineral fragments of numerous kinds in the presence of organic material and moisture constitutes a complicated system. Rainfall and evaporation, with seasonal and daily variations in the temperature, subject the soil surface to fluctuations so that, in general, the system must be regarded as dynamical, the principal "reactions" being a readjustment of the soil structure and a movement of the moisture. This paper is concerned with methods for studying the phenomena connected with this latter reaction.

Capillary and gravitational forces are always involved as factors determining the conditions of motion or of equilibrium of moisture in the soil. There is much evidence for believing that most soils are "wetted" by water, and hence the adhesive attraction of water molecules for soil grains and the cohesive attraction of one water molecule for another are suppressed to indirect relationship and need not enter into the analysis. Gravitational forces on the elemental

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[2] The author is deeply indebted to Prof. Willard Gardner for his kind assistance and encouragement. Appreciation is also expressed to Dr. D. S. Jennings for making available at various times soils and equipment of the soils department, and to Sterling J. Richards for assistance during the experimental work and in the preparation of this paper.

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particles are measured by the masses of the particles themselves, so there remains the single somewhat obscure force of capillarity with which to deal.

By making use of potential functions physicists have developed a dynamical method which has materially aided in studying the flow of heat and electricity. If we adopt this method we can remove many of the difficulties in the field of soil moisture by using what is known as the capillary potential function.[3] This function is simply an application of the well-known energy-potential theory. Capillary potential differences have the same relation to the flow of moisture in soil as voltage differences have to the flow of electricity in wires, or as pressure differences have to the flow of water in pipes. This latter is a rather close analogy because the capillary potential at any given point in the water between soil grains is numerically equal to the hydrostatic pressure at that point.[5]

Definitions and discussions of the capillary potential, involving more or less mathematics, are already to be found in available literature, but since potential functions have not been extensively used by workers in the agricultural sciences it has been thought worth while to present a discussion of the capillary potential without the use of detailed mathematical analysis. To aid in making clear the nature of this function the characteristics of the electrostatic, gravitational, and pressure potentials are briefly reviewed. The close relation between the capillary potential and the pressure potential (which in water is numerically equal to the hydrostatic pressure) makes it possible to introduce the capillary function in terms of more commonly used quantities.

This paper also presents some experimental data which show the relation between capillary potential and moisture percentage for several different soils, and attention is called to some of the ways in which capillary potential may be used.

DEFINITION AND CHARACTERISTICS OF POTENTIAL FUNCTIONS

ELECTROSTATIC POTENTIAL

In general, it may be said that potential functions [6] are more or less artificial creations of the mind, defined and used because they are helpful in studying and accurately expressing the processes of nature. In dealing with electricity we have come to regard potential as indispensable. Because the term "potential" is commonly associated with electricity, and because the electrostatic potential ts similar in so many ways to the potentials to be discussed in this paper, the characteristics of the electrostatic potential function are briefly reviewed.

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[3] The capillary potential as a magnitude to be used in the study of soil moisture was first introduced and defined by Buckingham (*2*) [4] in 1906. The methods then known for measuring this magnitude were rather slow and of uncertain accuracy, so the function was of little experimental use. It is an interesting coincidence that in 1906, the same year Buckingham submitted his article, Livingston (*13*) developed apparatus, called by him autoirrigators, which could be used as a quantitative means of controlling the capillary potential in soil. However, there seems to have been no correlation established between autoirrigators and the Buckingham potential function until about 15 years later, when it was pointed out by Gardner (*7*) and his associates that porous clay equipment could be used to measure the value of this function.

[4] Reference is made by number (italic) to "Literature cited," p. 741.

[5] The capillary potential in water is numerically equal to the hydrostatic pressure only when the units used are such that the density of water is unity.

[6] In a singly connected, conservative force field the potential at any point is the amount of work required to move a unit mass from an arbitrarily chosen point of zero potential to the point in question. If f as a vector is the externally exerted force on unit mass and ds an infinitisimal vector along the path, then the potential at any point B is, $W=\int_A^B f\,ds$ where A is the arbitrarily chosen point of zero potential. If there is no force in the field the potential will be constant throughout.

In case more than one force field exists in a region the potential for each field and the potential due to the resultant field or total potential is defined by the above relation. If equilibrium obtains, the forces are balanced and the total potential will be constant throughout the region. The potential at any point is independent of the mass present and is the work that would be required to move unit mass from the point of zero potential to the point in question.

If static electrical charges are accumulated at different places in a certain region there will be electrical forces exerted on other charges which are brought into that region and there is said to exist an electrostatic "force field." The field intensity, which is defined as the force on a unit positive charge, has a definite direction and magnitude at every point in the region. It is called a point function because every point in the region is characterized by the direction and magnitude of the field intensity at the point. Now, it is possible completely to represent this force field by means of another point function called potential. The potential is a function such that if its value is known for every point in the region then the direction and magnitude of the field force at any point can be calculated. The electrostatic potential at any given point is defined as the amount of work that must be done against the field forces in bringing a unit positive charge from some reference level to the point in question. The term "work," as here used, has its usual meaning. It is the product of a force times a distance. For example, if a force of 5 gm. moves a body a distance of 2 cm. in the direction of the force, then 10 gm. centimeters of work have been accomplished. Since potential is defined in terms of the work done in moving a unit charge in the force field, then the difference in potential between two points is simply the work required to move a unit charge from one point to the other. Hence, if the potential is known at two different points, then the average component of the field force acting on a unit charge in the direction of the line connecting the two points is simply the difference in potential divided by their distance apart, the force being directed from high to low potential. If we let V stand for the electrostatic potential, then for every point in an electrostatic field V will have a certain definite value. If V is everywhere the same, then it requires no work to move a charge from one point to another and the electric field intensity must be zero, i. e., there are no forces acting. If, however, V changes in value from point to point there will be a direction in which its space rate of change will be a maximum. This change in potential per unit of distance in the direction of the maximum rate of increase of potential is called the potential gradient and is designated by grad V. The field intensity E is everywhere equal in magnitude and opposite in direction to the gradient of V, or

$$E = -\text{grad } V \tag{1}$$

This relation is especially important.

As we proceed, it should be noticed that there is a striking analogy to the electrostatic case between both the way in which the gravitational and pressure potentials are defined and the way in which the gravity and the pressure field force is related to the potential gradient.

GRAVITATIONAL AND PRESSURE POTENTIALS

Bernouilli's equation, which is discussed in almost every college physics textbook, expresses the relation connecting the gravity energy, the kinetic energy, and the pressure in a moving fluid.[7] It may be written as follows:

$$gh + p/\rho + \tfrac{1}{2}v^2 = K \tag{2}$$

where, in the centimeter-gram-second system of units, h is the height in centimeters of a point above a fixed level; g is the acceleration of gravity, 980 cm. per second per second; p is the pressure in the fluid at the point expressed in dynes per square centimeter (in the centimeter-gram-second system the dyne is the unit of force; 980 dynes = 1 gm.); ρ is the density of the fluid in grams per cubic centimeter; v is the velocity of the fluid at the point in centimeters per second; and K is a constant.

We shall first consider systems which are at static equilibrium. Under this condition the velocity is zero and equation (2) becomes,

$$gh + p/\rho = K \tag{3}$$

In applying this equation we shall deal only with the potential and force relations within the region occupied by the liquid. The first term is the expression for the gravitational potential at the point under consideration. It is the work, in dyne centimeters, that would have to be done against the gravity field force in order to raise 1 gm. of matter to a height h centimeters above a gravity reference level. For the systems we shall here consider, the free flat liquid surface will be used as the reference level for the gravitational potential. Let the gravitational potential be designated by ϕ. Then from (3) the value of ϕ at any point in the region is

$$\phi = gh \tag{4}$$

where h is the vertical distance of the point from the free flat water surface level. If the point is above this surface, then h will be taken as a positive quantity. For points below the water surface, h will be negative. (This convention as to the algebraic sign of h will be used in subsequent formulas in which h is used.)

Equation (4) shows that the gravitational potential is proportional to the vertical distance from the reference level and that it increases with the height. This means that vertically upward is the direction of the potential gradient. The gravity force per unit of mass, F_g, is vertically downward and is equal in magnitude to the space rate of change of the potential, i. e., $F_g = -\text{grad}\ \phi$.

The second term in equation (3), p/ρ, may also be interpreted as a potential, defined for every point throughout the region occupied by the liquid. This is the potential due to the internal stress of the material and is called the pressure potential. It has been designated by the letter π [8]. At any point in the liquid,

$$\pi = p/\rho \tag{5}$$

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[7] Bernouilli's equation is valid for steady irrotational motion of a uniform frictionless fluid.

[8] The more general expression (*17, p, 149*) for the pressure potential at a point B is, $\pi = \int_A^B dp/\rho$ where A is the arbitrarily chosen point of zero pressure potential. This expression holds for both positive and negative pressures and is valid through varying densities. When ρ is constant the above integral reduces to p/ρ where p is the difference in pressure between A and B.

When dealing with the gravity potential, the work done in raising a unit mass against gravity may be thought of as being stored in the mass as gravity potential energy, but the term "pressure energy," which is ordinarily associated with the p/ρ term in Bernouilli's equation, is inaccurate and should be avoided (*9*).

where p is the value of the pressure at that point. In the case of water, ρ is equal to unity, and hence for this liquid π is numerically equal to the pressure. It is a matter of common observation that if a horizontal tube is filled with water and there is a difference in the pressure at the two ends, the water will flow from the high to the low pressure end. Let us consider a unit cube of liquid in such a tube in which there is a difference in pressure of 1 dyne per square centimeter per unit of length in the horizontal direction. When this condition exists the pressure force on one end of the cube will be 1 dyne greater than the pressure force on the other end and there will be a resultant force of 1 dyne per cubic centimeter, tending to move the water in the direction of the decrease in pressure. It should be noticed that under the conditions of this example the pressure potential has a gradient of one potential unit per centimeter in the direction of the increase in pressure. Hence, the property of the electrostatic potential set forth in equation (1), is also a property of the pressure potential, i. e., the field force, F_p, due to the pressure in the liquid is equal to the negative potential gradient or, $F_p = -\text{grad } \pi$.

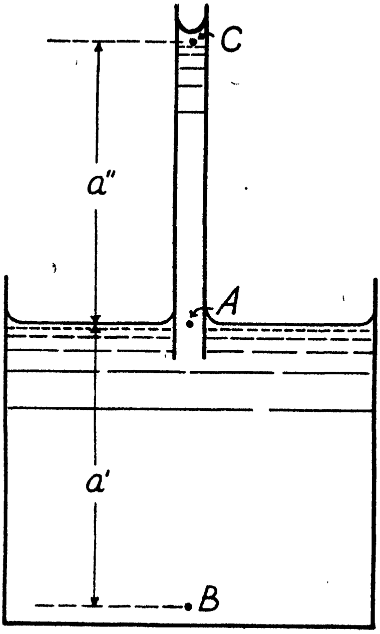

FIG. 1.—Capillary tube dipped into a free water surface

In working with potential functions the potential gradients and potential differences are the significant quantities, and these are independent of the place chosen as the reference level or place of zero potential. That is, if a different reference level were chosen the potential would take on a new value for every point, but the size and direction of the potential gradient at any point or the difference in potential between any two points would remain unaltered. For the systems considered in this paper, and for soil-moisture work generally, it will be found convenient to choose the same reference level for both the gravitational and the pressure potential, i. e., the region of zero hydrostatic pressure or the free flat water surface.

With the pressure at the free flat water surface chosen as the zero reference level for π, then the value of π at all other places is simply equal to the difference in pressure between the flat water surface and the point in question. If at a given point the pressure is less than at the surface, then π is negative, and vice versa.

Let us consider a system in which both the pressure and gravity force fields are acting. If a capillary tube is dipped into a free surface (fig. 1) the water will rise in the capillary tube and come to rest. When the water is at static equilibrium the forces on each little element of water must be balanced. Hence, there must be some force acting in the liquid to neutralize the force of gravity. The forces that are acting may be derived by considering the potentials which are involved. Since the water is at rest, equation (3) must hold. Consider first the gravitational potential. For all points at the same level as A, which is at the level of the free flat water surface, $\phi = O$. At all other places in the liquid $\phi = gh$. That is, at C, $\phi = ga''$, and at B, $\phi = ga'$. The gravitational potential at this latter point is negative because a' is negative. From the expression $\phi = gh$, it is easily seen that all points in the same horizontal plane have the same gravitational potential. The potential gradient is always perpendicular to the equipotential surface, and in this case is vertically upwards. This is the opposite direction to gravity which is the field force of this potential.

$F_g = -\text{grad } \phi = g = 980$ dynes $= 1$ gm. $=$ gravity force per unit of mass.

According to the convention here adopted, the pressure potential π is also zero at all points at the same level as A, where the hydrostatic pressure is zero. At other levels $\pi = p/\rho$.

The expression for the hydrostatic pressure in a liquid is $p = -\rho gh$, where ρ, g, and h have the same meanings as previously used. Substituting this value for p in the expression above gives $\pi = -\frac{\rho gh}{\rho} = -gh$. Therefore, at the point B, $\pi = -ga'$. Because a' is negative the value of π at B is positive and is numerically equal to the hydrostatic pressure in the liquid at that level. At the point C, $\pi = -ga''$. π is negative at C because g and a'' are both positive quantities. The negative sign indicates that the water at the point C, just under the meniscus, has a negative pressure, or that it is under tension. Thus, the pressure and the pressure potential are positive at B, decrease to zero at A, and become more and more negative with increasing height in the capillary tube.

The equipotential surfaces of the pressure potential are horizontal because all points at the same level have the same hydrostatic pressure. However, the gradient of π is vertically downward because that is the direction in which π increases. The field force due to pressure difference is in the opposite direction to the gradient, and hence acts vertically upward. Pressure and gravity are the only force fields acting in a system such as Figure 1, so if the water is in static equilibrium the pressure and gravity forces on each little element of water must be balanced. There must be at every point a force due to a pressure gradient which is just equal and opposite to the force of gravity.

If at every point in the region just considered we add together the value of π and ϕ we shall have a new point function which we shall call Φ, the total potential.

$$\pi + \phi = \Phi \tag{6}$$

Φ has the same properties as the two potentials of which it is composed. Grad π is a measure of the forces set up due to the internal stress of the water, grad ϕ is a measure of the force due to gravity, while grad Φ is a measure of the resultant of both of these forces acting on a unit of mass at any given point. Since the system we have just considered is at static equilibrium the resultant of all the forces acting on each little particle of the liquid must be zero, and hence there must be no gradient of the total potential. This is the condition set forth by equation (3), and it is easily seen to be true, for

$$\pi+\phi=-gh+gh=\Phi=0 \tag{7}$$

everywhere in the system. At the water surface $\pi=0$, $\phi=0$, and therefore $\Phi=0$. Everywhere else π is just the negative of ϕ, so that they add up to zero. Thus the space occupied by the water in the system is an equipotential region for Φ, and because of the reference level convention that has been adopted, Φ is everywhere equal to zero. When this condition obtains the gravity and pressure forces are just balanced and, neglecting friction, a very slight impulse would be sufficient to move a little mass of water anywhere in the system.

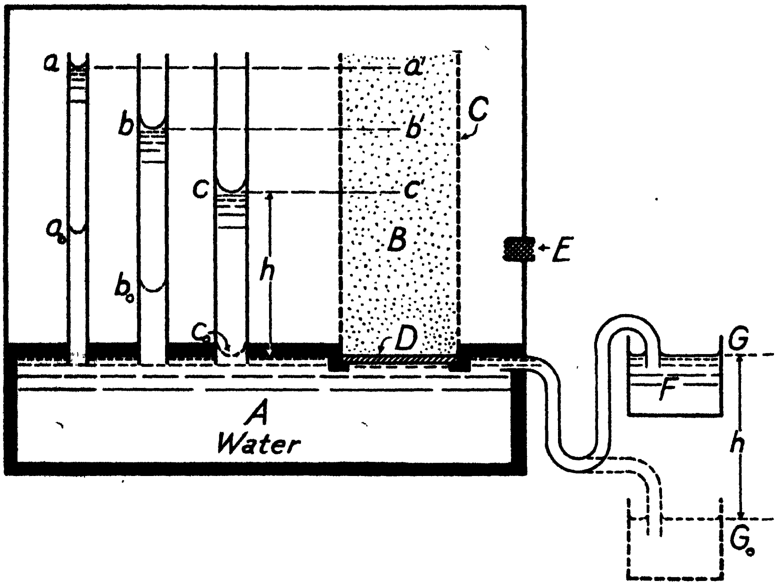

Fig. 2.—A system to aid in studying the moisture relations in a soil column. See text for complete explanation

As an example more closely related to the soils problem let us consider the forces and potentials in the system illustrated in Figure 2. A is a water tank fitted with capillary tubes as indicated. B is a column of soil supported by a cylindrical sieve, C, and closed at the bottom with a porous clay plate, D, readily permeable to water. The vertical tubes are inclosed in a case which is provided with a porous plug opening, E, to maintain atmospheric pressure within, but to prevent the loss of water vapor from the chamber. When the tank, A, is filled, and the water in the auxilary tank, F, is so adjusted that the free water surface is maintained at the level, G, then water will rise in the capillary tubes and soil column and will reach an equilibrium distribution, provided the temperature everywhere in the system is maintained uniform.

To simplify the problem, let us here assume that pure water and washed soil are used. When the system has come to equilibrium under isothermal conditions, let us say that the water has risen in the capillary tubes to the heights a, b, and c, respectively. These heights will be determined by the radii of the tubes and the surface tension of the water according to the formula $h = \frac{2T}{rg}$ where g is the acceleration of gravity, r is the radius of the tube, and T is the surface tension of the water (c. g. s. units implied).

The configuration of the region occupied by the water in the soil is not definitely known, but it is probable that in moist soils the water forms a continuous and connected configuration which spreads out in thin films over the surface of the soil grains and collects into wedges where the grains are in contact or are very close together. It is the relation between the potentials and the forces in this water configuration that we are particularly interested in.

In Figure 2, all points in the liquid at the same level as G—the level of the free water surface—have $\pi = 0$, $\phi = 0$, and hence, $\Phi = 0$. We shall consider the pressure and gravity force relations in the soil-water configuration quite analogous to those existing in a capillary tube. The forces and potentials may be analyzed by the same method that was used for the system shown in Figure 1, and at equilibrium—according to equation (3)—Φ must have the same value everywhere in the region occupied by the water.

It is well known that the vapor pressure of a liquid depends on the curvature of the liquid surface (*3*). When equilibrium exists in the system of Figure 2, the water surfaces in the soil at a' must have the same curvature as the capillary meniscus at a, for if such were not the case there would be a difference in the vapor pressure at the two points. This would result in distillation and in the motion of water in a cycle, which, according to the second law of thermodynamics, is impossible under isothermal conditions.

As was shown above, π has the same value for all points in the capillary tubes or soil column which are at the same height above the free water level, G. This means that the pressure in the liquid at all these points is the same. That this is the case may be indicated by the following different method of reasoning. When equilibrium obtains, all the liquid surfaces at the same height have the same vapor pressure and hence the curvature of the air-water surfaces in the soil at a', b', and c' is the same as that at the corresponding heights, a, b, and c, in the capillaries. (Pure water and constant temperature are assumed.) The difference in pressure on two sides of a curved liquid surface is given by the well-known formula

$$p = T\,(1/r_1 + 1/r_2) \tag{8}$$

T is the surface tension of the liquid in dynes per centimeter and $(1/r_1 + 1/r_2)$ is the expression for the total curvature of the surface.[9] The surface tension and curvature have the same values at both a and a', and since the pressure in the vapor at a and a' is practically equal to the pressure in the water at the flat surface (neglecting the pressure difference in the vapor between a and D due to the weight of the column of gas between these two points), then the value of the pressure difference in the liquid between the points a and D, and a' and D, is given by equation (8) and is numerically equal to π. Hence, the value of π when determined in this manner will be the same for all points at the same level because the surface tension and curvature are the same at all these points.

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[9] r_1 and r_2 are the radii of curvature of the two curves formed by the intersection of two planes (principal planes) at right angles to each other and passing perpendicularly through the water surface at the point where it is desired that the curvature be known.

The above reasoning holds, of course, only for pure water in an isothermal equilibrium system. However, if we still retain the assumption that ρ is equal to unity, then the value of π in soil water is correctly given by equation (8), even if isothermal equilibrium conditions do not obtain.

CAPILLARY POTENTIAL

The preceding discussion of potentials was given primarily as a preparation for introducing and defining the capillary potential, which we shall designate by the letter ψ. The value of this function at a given point in moist soil was originally defined by Buckingham (*2*) as the work that would have to be done against the "capillary field force" in transferring a unit mass of water *from the soil to free water* at zero hydrostatic pressure. Subsequent writers (*4*, *8*), however, have defined this function as the work done against the capillary field force in moving unit mass of water *from the flat water surface to the point in question.* This definition gives ψ the opposite algebraic sign to that used by Buckingham. This latter definition is more in accord with the way potentials are ordinarily defined and is the definition that will be used in this paper.

Just what is meant by "capillary field force" is rather obscure. If we interpret this expression, as Buckingham indicated, as simply the mechanical force involved in the attraction of moist soil for water, then we may consider it as the force set up in the soil water configuration due to pressure differences or due to a pressure gradient. When this is understood, it is easy to see that the capillary potential and the pressure potential are the same. That is,

$$\psi = \pi \tag{9}$$

No one questions the significance of the pressure potential when studying the flow of water through pipes or the motion of ground water where the soil is saturated, but in soil which is not saturated, the pressure in the water is negative, and for studying the flow of water under such conditions neither the pressure potential nor the capillary potential has been very much used.

That the pressure is negative makes little difference. The potential functions should be as useful in soil-moisture work as they have been in dealing with electricity or heat. Failure to use capillary potential when making a detailed study of the flow of water through soil is almost as inconsistent as not taking advantage of the well-known equation of Fourier [10] when studying the flow of heat at temperatures below zero, because the distinction between positive and negative pressures is almost as arbitrary as the distinction between positive and negative temperatures.

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[10] Fourier's equation for the flow of heat will be discussed further on in the paper.

Because of its descriptive fitness, capillary potential will be used for regions such as moist soil where the hydrostatic pressure is negative; but it should be understood that the two terms, capillary potential and pressure potential, are interchangeable. The place of zero hydrostatic pressure has been chosen as the reference level. The capillary potential will therefore be negative in moist soil or wherever the hydrostatic pressure is negative.

MEANS OF CONTROLLING CAPILLARY POTENTIAL

With the proper arrangement of porous clay apparatus the capillary potential in soil which is in contact with the porous surface may be accurately controlled. Let us refer again to Figure 2. When the water surface in the auxiliary reservoir is at the level G, the meniscus in the largest capillary tube, *c*, is at a height *h* centimeters above G. This height corresponds to a capillary potential of $\psi = -gh = -980h$ dyne centimeters per gram. (This is equivalent to a tension of 980 *h* dynes per square centimeter in the water at *c*.) Now if the reservoir, F, is lowered so that when the system again comes to equilibrium the new free water level is *h* centimeters lower than before, then the meniscus in each capillary tube will be depressed by this amount. Under the new conditions the curvature of the water films in the soil on the porous plate is the same as that which previously obtained at the height *h* centimeters above the plate, because this curvature is the same as the curvature of the water meniscus now at C_0. The value of ψ in the soil on the plate is negative and is numerically equal to g times the vertical distance down to the new free flat water surface. It is evident that with the initial conditions (free water level at G) the curvature and pressure relations obtaining at any point in the soil column would be unchanged, if, at some position lower in the soil column, a horizontal porous plate were interposed, the plate being mounted in such a way that its under side could be kept in contact with a column of water reaching down to the free water surface. When using the centimeter-gram-second units, the capillary potential in soil which is in equilibrium with water through a porous plate is numerically equal to the difference in pressure in the water under the plate and the atmospheric pressure. This pressure difference may be determined by means of a water or mercury manometer. With porous clay apparatus of sufficient strength and fineness of porosity, pressure differences as high as 1 atmosphere may be maintained in this manner.

In the system illustrated in Figure 2, the conditions of uniform temperature, pure water, and static equilibrium throughout the system were assumed. These conditions of course would be impossible to attain in any actual soils experiment. Nevertheless, the methods used in this example for deriving the capillary and gravitational field forces from their corresponding potentials may be quite generally applied.

When the soil moisture is not at static equilibrium with free water, then the relation $\psi = -gh$ no longer holds. For liquids of constant density ψ is defined by equation (5), i. e.:

$$\psi = p/\rho \tag{10}$$

For soil-moisture work generally we may assume ρ equal to unity[11] because the errors involved in measuring ψ will be greater than those introduced by this assumption. Equation (10) then becomes

$$\psi = p \tag{11}$$

where p is simply the pressure difference between the reference level and the point in question (c. g. s. units implied).

APPARATUS FOR MEASURING CAPILLARY POTENTIAL

When the tension in the soil water is less than 1 atmosphere, the value of ψ in a given soil may be accurately determined by a direct method. The apparatus shown in Figure 3, called a capillary potentiometer, has been used in this laboratory for various soil experiments. It consists of a porous cup, A, sealed onto a glass tube which is connected by heavy pressure tubing to a mercury manometer, B. The porous cup and the manometer are filled with water, a rubber stopper is inserted at C, and the cup is imbedded in soil. When water moves from the cup into the soil, the mercury rises in the manometer and the pressure within the cup is reduced. This process continues until the soil water and the cup water have the same pressure. The difference between this pressure and atmospheric pressure is numerically equal to the capillary potential of the soil and may easily be determined by properly reading the manometer.

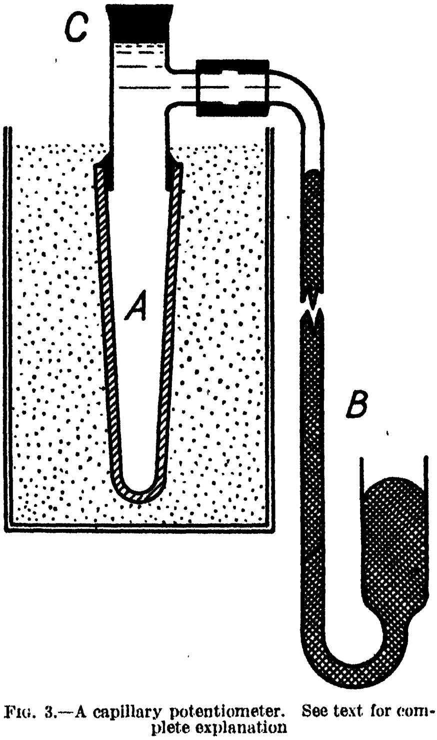

FIG. 3.—A capillary potentiometer. See text for complete explanation

When using equations (2) and (3) the pressure difference should be expressed in dynes per square centimeter and would correspond to a potential in dyne centimeters per gram (work per unit of mass). However, for practical purposes these units give large numbers with which to deal. The capillary potential may be conveniently expressed in terms of the length of the water column which the tension in the soil water would be able to support. If the length of the water column is given in centimeters, then the potential units would be gram centimeters per gram and would correspond numerically to the tension in the liquid expressed in grams per square centimeter.

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[11] ρ is sometimes used to represent the mass of water per unit aggregate volume of the soil (*5*, *6*, *8*), but in this paper ρ is used only to represent the mass per unit volume of water or soil solution.

FACTORS DETERMINING THE CAPILLARY POTENTIAL

As was seen from considering Figure 2 and the definition of ψ, the *equilibrium value* of the capillary potential in a soil column at a given height above a free water level is a constant, independent of soil structure, temperature, or dissolved material in the soil solution. The percentage of moisture in the soil at this height, however, varies with all of these factors.

ψ is a magnitude determined by the pressure in the water. Because of our choice of the pressure reference level, water is said to be under tension when its pressure is less than atmospheric pressure. (The pressure in water at a free flat water surface is the same as atmospheric pressure.) The difference in pressure between the water under the little curved surfaces of the soil solution and the atmosphere is given by the formula (8) $p = T\left(\frac{1}{r} + \frac{1}{r_2}\right)$. If we could measure the surface tension and surface curvature of soil water, then we would be able to calculate the value of ψ at any point. This would be unnecessary for certain values because, as was seen earlier, the potential can be measured directly by means of the capillary potentiometer.

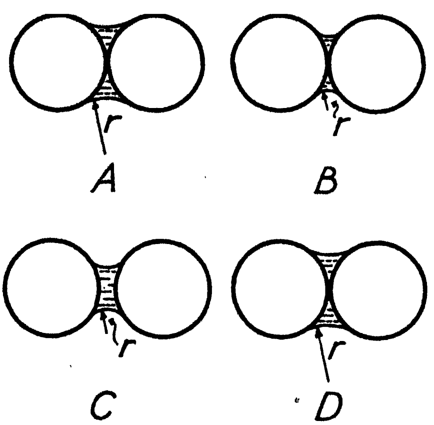

FIG. 4.—Water wedges between spherical soil particles. Decreasing the amount of water between articles (A) increases the curvature of the water surface (B); pressing the particles closer together (C) decreases the curvature (D)

Let us now see how the value of ψ in a given *isolated* mass of moist soil is affected by the temperature, moisture content of the soil, dissolved material in the soil solution, size of soil particles, and state of packing. Any change which increases the surface tension or the curvature will decrease ψ, and vice versa.[12]

For a mass of soil with a certain state of packing and water content, decreasing the temperature or increasing the amount of dissolved salts in the soil solution will increase the surface tension and hence decrease ψ. The other factors—moisture content, size of soil particles, and state of packing—will affect the value of ψ through their effect on the curvature. As is indicated in Figure 4, A and B, if the amount of water collected between two soil grains is decreased, there will be an increase in the curvature of the water surfaces, hence a decrease in ψ. That is, the drier the soil, the more negative the capillary potential will be. Also, if equal weights of a fine and a coarse soil have the same moisture percentage, the fine soil will have more surface and more contact points between soil particles. There will be less water collected at each of the contact points, and it would be expected that the fine soil will have a lower potential than the coarse soil even though their moisture percentages are the same.

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[12] The magnitude of ψ will be spoken of in the algebraic sense. That is, −5 is greater than −10, or, as a further example, ψ *increases* if it changes successively from −10, −5, 0, 5, 10, and so on.

The compactness of the soil also influences ψ. Referring again to Figure 4, C and D, if two particles of soil are pushed closer together the curvature of the water surface will be decreased. If comparatively dry soil is sufficiently compressed, water will run out under the force of gravity. During this process of compressing the soil (until the water is just ready to flow out) the moisture percentage, expressed on the dry-weight basis, remains unchanged, but ψ changes from a low negative value to zero.

For a magnitude that has as many influencing factors as ψ it is desirable to hold all the factors constant except one and see how the magnitude varies when this one factor is changed. In studying the capillary potential the moisture percentage of the soil seems to be the factor best suited for this purpose. If the contact angle between water and the soil particles is zero, we should expect that for a given soil, having a certain temperature and state of packing, there would be a certain definite value of ψ for each value of the moisture percentage. All the experimental data thus far collected seem to support this conclusion.

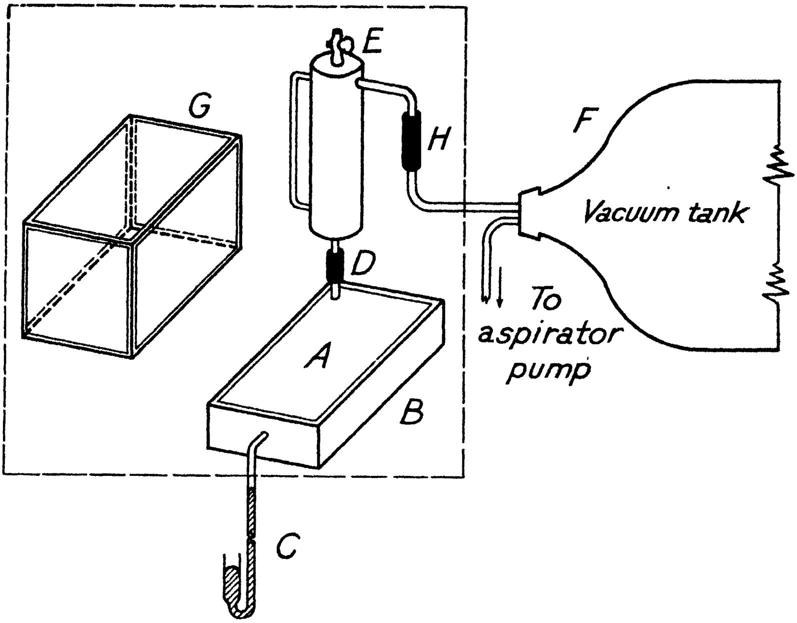

FIG. 5.—The arrangement of the apparatus for determining the capillary potential in soil. See text for complete explanation

MOISTURE PERCENTAGE EXPERIMENTAL DATA

As was pointed out earlier, the value of ψ in soil which is in equilibrium with water through a porous plate is numerically equal to the difference in the pressure in the water under the plate and atmospheric pressure. By making use of this fact the apparatus shown in Figure 5 served to determine the moisture percentage-capillary potential curves for several types of soil. The porous plate, A, was specially made for the purpose, and is 9 inches long, 5 inches wide, and about one-fourth inch thick. It is mounted in the cast aluminum case, B, and an air-tight seal is effected by means of plaster of Paris and a soft wax. The mercurial manometer is connected at C. A ⅜-inch copper tube is sealed in to the porous plate at D and a vacuum rubber connection made to the water reservoir, E. To put the apparatus in operation the manometer is supplied with mercury and the capillary cell and reservoir are filled with water. The corner of the plate at D is slightly raised so that any air which accumulates within the cell may pass upward through the water in the reservoir. By means of an aspirator pump the pressure in the vacuum tank, F, is reduced until the mercury stands at the desired level in the manometer. The soil for the experiment is then spread on the plate to the desired depth and covered with the glass cage, G. The soils used were air-dried and reduced to a fine powder by means of a small hand grinder.

Four of the complete units shown in Figure 5 were used. That part of the apparatus within the dotted line was mounted in an air thermostat, the air being well stirred by an electric fan. For the data here presented the temperature was maintained at 16.1° C. During the course of the experiments, continuous thermographic records were kept, and except for short intervals when samples were taken, the temperature fluctuations were less than one-tenth of a degree centigrade. The interior of the case was kept dark to avoid the temperature effects of light observed by Linford (*12*). When the apparatus was ready the plates were covered with a ½-inch layer of soil, the glass cover cages installed, and the thermostat closed. The dry soil absorbs water from the porous plate until the soil moisture is in pressure equilibrium with the water under the plate. Since the maximum distance the water moves through the soil is one-half inch, this equilibrium is rapidly approached. The carboy pressures were adjusted so that the mercury levels in the manometers remained constant from two to four days to permit the soils to reach equilibrium. At the end of this time 40 to 50 gm. soil samples were taken from the plates and the pressures set at some new values. The moisture determinations were expressed as percentage of water for dry weight of soil. The data for four different soils are given in Table 1 and Figure 6.

TABLE 1.—*The moisture percentage and capillary potential values of four soil samples*

[The moisture is expressed as percentage of water for dry weight of soil and the corresponding capillary potential is given in gram centimeters per gram]

Bennet sand		Greenville loam		Trenton clay		Preston clay	
Moisture	Potential	Moisture	Potential	Moisture	Potential	Moisture	Potential
20.9	4	39.6	5	67.3	4	66.5	6
11.7	26	34.2	20	52.5	27	60.4	22
9.5	54	30.2	55	45.2	53	57.8	31
7.6	82	27.0	84	43.0	54	51.5	58
6.9	110	22.8	144	40.5	82	46.3	85
6.2	150	20.5	222	38.0	103	42.4	143
5.7	240	19.4	269	33.0	125	40.2	196
5.3	348	19.0	304	30.7	216	33.0	287
5.1	301	17.5	371	28.4	286	31.6	438
4.9	427	17.0	429	27.2	380	31.2	362
4.8	452	16.4	489	26.2	467	29.4	527
4.7	492	15.5	556	26.2	558	29.0	650
4.1	684	15.2	656	24.5	800	28.7	729
4.0	596	15.0	715	24.3	612	28.5	664
3.9	692	14.6	796	24.2	735	28.4	611
3.7	801	--------	--------	24.0	745	28.3	784
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Since ψ is negative, the fourth quadrant is used for plotting. The soils used were: A, a loose sandy loam; B, a clay loam; C, a heavy subsoil clay; D, a fine clay from Preston, Idaho. (The porous plates were made from Preston clay.) The potential units used in plotting are gram centimeters per gram, and correspond numerically to the length in centimeters of the water column which the tension in the water would be able to support.

If large soil tubes 26 feet high were filled with these soils at the temperature and state of packing here used, and with their lower ends dipped into free water were allowed sufficient time for the water to come to static equilibrium, then the distance above the flat water level would correspond numerically to the potentials, and the moisture percentages at any height up to 800 centimeters (26 feet) for the different soils could be determined from the curve.

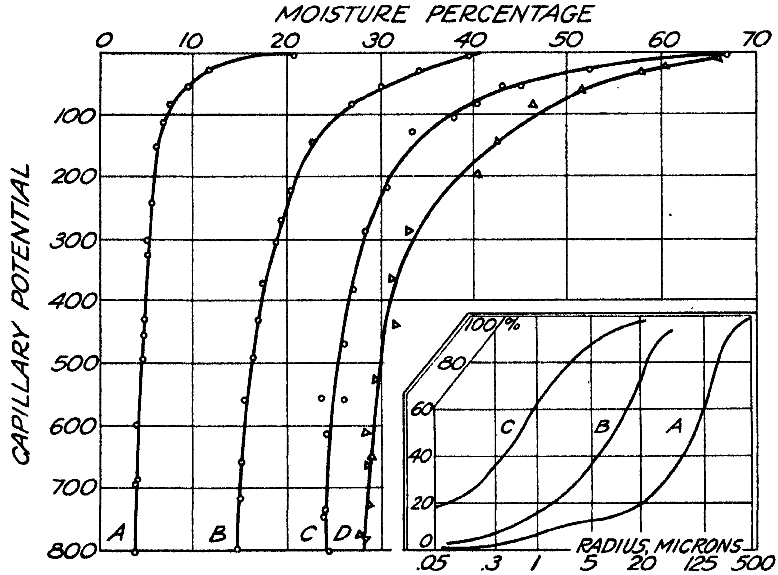

FIG. 6.—Capillary potential-moisture curves for: A, Bennet sandy loam No. 629 from the Uinta Basin; B, Greenville clay loam from the college farm; C, Trenton subsoil clay No. 1232; D, clay from Preston, Idaho. The inset shows the mechanical composition of soils A, B, and C, the ordinates showing the percentages of material of smaller radius than the corresponding abscissas

The mechanical analyses for soils A, B, and C, as given by Thomas (*19*), are shown in the inset, Figure 6. The effect of the size of the soil particles on the moisture content of soils at any fixed potential is very evident.

Because of the short distance the water has to move and the relative ease of duplicating the soil structure, the apparatus here described for determining the relation between ψ and moisture percentage has some advantage over methods previously employed. The capillary conductivity seems to be less for clay than for sand, and the greater scattering of the experimental points from the smooth curves for the clays C and D indicates that for these soils two to four days was not sufficient time for the water to reach equilibrium. Another source of error for this method lies in the fact that the mercury levels in the capillary cell manometers fluctuate with the atmospheric pressure. Continuous barographic records were kept to aid in compensating for barometric changes. For the low potential points the manometer (fig. 5, C) was replaced by a rubber tube reaching down to free water at the desired level. With this arrangement barometric fluctuations do not influence the value of ψ in the porous plate.

APPLICATION OF CAPILLARY POTENTIAL THEORY

MOISTURE GRADIENT EXISTS

From the discussion given with Figure 2 it is clear that when moisture in a vertical soil column is at static equilibrium with a free water table, there must be in the water a gradient of the capillary potential having such direction and magnitude that the capillary or pressure forces just balance gravity. This means that the tension in the soil liquid must increase with the height above the water table. If the surface tension remains sensibly the same throughout the column, then, according to equation (8), the curvature of the air-liquid surfaces of the soil solution must increase with the height above the water table. As is indicated in Figure 4, this may be accomplished by having a looser state of packing, or by a decrease in the moisture content with increasing height above the water table. Experiments by King (*10*), Buckingham (*2*), Israelsen (*8*), and others indicate that the moisture gradient is necessary in order to make possible the proper capillary potential gradient for static equilibrium.

That this moisture gradient will exist under equilibrium conditions is still reluctantly accepted by some soils workers (*20*). The fact that this moisture gradient is not common in the field is easily explained. There is abundant evidence to show that the motion of water in dry soils is very slow. Under field conditions the seasonal and diurnal fluctuations of temperature, evaporation, precipitation, irrigation, and ground water, or drainage conditions make it unlikely that equilibrium is very closely approached in any actual case. Even if equilibrium were attained, the curves (fig. 6) indicate that for a deep-water table in the coarser soils the moisture gradient is so small that precise moisture-determination methods would be required for its detection. If static equilibrium were attained in a loam soil such as B, Figure 4, then a moisture gradient somewhat similar to that shown by the curve would be expected.

SOILS NOT AT MOISTURE EQUILIBRIUM—CAPILLARY FLOW

Thus far we have considered only soil-water systems which were assumed to be at static equilibrium. The preponderance of evidence indicates that for actual cases in the field this condition is exceptional. Soil moisture is generally in motion. It is in the study of the dynamics of capillary flow that the potential theory is particularly useful. The reader is especially referred to the lucid nontechnical discussion given by Buckingham (*2*) on this phase of the subject; also to the more recent work of Gardner (*5, 6*) and Israelsen (*8*). This application of the potential theory is of prime importance because it offers a direct method for getting at the fundamental factors which determine capillary flow. Because the above-cited literature is available this phase of the subject will be reviewed but briefly in this paper.

We have seen that grad Φ, the gradient of the total potential, is a measure of the total "water-moving" force or the net resultant force (per unit of mass) which tends to produce motion of the soil water. Darcey's equation states that the velocity of the water is proportional to the force which is producing the motion, hence we may write,

$$v = -K \text{ grad } \Phi \tag{12}$$

where v is the velocity and K is the proportionality constant. Both the velocity and grad Φ are vector quantities because they have definite directions as well as magnitudes. Grad Φ is in the direction of the greatest rate of increase of potential. Since the water moves in the opposite direction the negative sign is used in the equation.

Equations similar to (12) are very useful in studying the flow of heat and electricity. Pointing out some of these similarities will aid in understanding equation (12) and help to attach a physical meaning to the constant K.

Fourier's law for the flow of heat is

$$q = -C \text{ grad } \theta \tag{13}$$

where q is the velocity of flow, or the quantity of heat crossing unit area perpendicular to the flow in unit time. The temperature, represented by θ, is a point function and may be spoken of as the thermal potential. Grad θ is the change in temperature per unit of distance in the direction of the greatest rate of increase in temperature. C, called the thermal conductivity, is a constant for a given kind of material. It is the amount of heat that would flow through a unit cube of the material in unit time if two opposite faces had a temperature difference of 1 degree. Qualitatively every one knows that a bucket of hot water will cool off faster in cold air than in warm air. This is because there is a greater temperature difference causing the heat to flow out through the walls of the bucket. Also the water would cool faster in a metal bucket than in a wooden one, because metal is a better heat "conductor." Equation (13) is a quantitative expression of these relations and simply states that the conductivity times the temperature gradient is equal to the heat velocity, or the quantity of heat transferred across unit area (perpendicular to the motion) per unit of time.

A similar expression has been found to hold for the electrical case. Ohm's law for steady electrical currents in metallic conductors may be written,

$$i = -C' \text{ grad } V \tag{14}$$

where i is the current density, C' the specific conductivity, and grad V is the change in potential per unit distance.

From analogy with (13) and (14) we may call K in (12) the specific capillary conductivity and define it as the amount of water which will flow in one second across a unit area in the soil, perpendicular to the direction of flow, when Φ changes at the rate of one unit per centimeter. However, the above analogy should not be pushed too far.

The thermal and electrical conductivities for a given piece of material are independent of the strength of the current and are in general only slightly dependent on the temperature and other outside influences, but the capillary conductivity K will depend on the kind of soil, its moisture content, and state of packing.

It is true, of course, that before equation (12) can be usefully applied in actual problems this transmission constant will have to be studied for a large number of soils under different conditions, but this procedure seems to be our best chance for reducing the phenomena of capillary flow to a quantitative basis. This method has met with splendid success with heat and electricity and it should not be any more difficult to make reliable capillary potentiometers than it is to construct good thermometers or voltmeters.

For soil-moisture conditions where the tension in the liquid is not greater than 1 atmosphere, porous clay apparatus offers a convenient means for measuring and studying the capillary transmission constant. One experiment has been attempted in this laboratory with this end in view, but the experiment was unsuccessful because the apparatus was not of the proper design. However, with a short column of soil properly arranged between two capillary cells such as those shown in Figure 5, it should not be difficult to obtain a steady and measurable capillary flow from one cell to the other. The capillary potential gradient would be determined by the pressure difference in the water in the two cells and the moisture content of the soil could be determined from the capillary potential-moisture percentage curve for the soil.

The gradient of Φ may be computed from the relation

$$(15) \qquad \text{grad } \psi + \text{grad } \phi = \text{grad } \Phi$$

Grad ϕ is a constant numerically equal to g. Potential gradients are vector quantities and must be added according to the parallelogram law which is used for the addition of forces. The resultant direction and magnitude of grad Φ determines the direction and velocity of the soil-water movement. When grad Φ and the velocity of flow, v, are known, the conductivity, K, may be evaluated by substituting in equation (12).

Three common cases of capillary water motion are: (*a*) Movement of precipitation or irrigation water downward through a comparatively dry soil, (*b*) motion of water upward from a saturated level or water table, and (*c*) motion of water in a horizontal direction. In case (*a*), grad ϕ and grad ψ are such that both the gravity and capillary forces tend to move the water downward. This downward motion will continue (provided there is no surface evaporation) until the soil is drained dry or until the soil moisture comes to equilibrium with an impermeable layer or a saturated water table. In case (*b*), the gradient of the capillary potential ψ has such direction and magnitude that there is a resultant upward "water-moving force." If there is no evaporation or transpirational loss of water from the surface the state of static equilibrium will ultimately be reached. However, when surface loss occurs, the capillary potential gradient is maintained and the upward flow continues. When the moisture content of soil is low the capillary conductivity is very small and it requires large potential gradients (comparatively wet soil in contact with the dry) to produce an appreciable flow. Movement of water in a horizontal direction, case (*c*), is due entirely to a horizontal component of grad ψ. If undisturbed, the motion will continue until all points at the same level have the same potential.

THE AVAILABILITY OF SOIL MOISTURE TO PLANTS

The problem of the availability of soil moisture to plants becomes very much simplified when considered in terms of the potentials. The term "availability" involves two notions, namely, (*a*) the ability of the plant root to absorb and use the water with which it is in contact, and (*b*) the readiness or velocity with which the soil water moves in to replace that which has been used by the plant.[13]

CASE (A)

For a plant which is growing in moist soil it is necessary for the root to absorb water from the little films and wedges among the soil particles. If the tension in the soil water is sufficiently high it is reasonable to suppose that the osmotic forces acting through the root-hair membranes may not be great enough to enable the plant to use even that soil water which is in contact with the root. The capillary potential is a measure of this tension in the soil water and, hence, should be a good index to the "security" with which the water is held by the soil. The term "security" as it is here used relates only to the pressure differences involved. Of course, it is possible for a plant to be in a wet soil and still be wilted and suffering from physiological drought if the salt content of the soil solution is sufficiently high. The effect of dissolved material on the availability of soil moisture which is in contact with the root could be expressed by adding to ψ the value of the osmotic potential as used by Linford (*12*). For a more complete index to soil-moisture condition it would be possible to use thermodynamic potential or free energy (*11*).[14] However, the capillary potential as here defined will be found useful over a wide range of soil-moisture conditions.

CASE (B)

The other factor in the availability of soil, namely, the rate at which water flows in toward the root, is concisely expressed by equation (12). The rate of flow across unit area perpendicular to the direction of movement is simply the conductivity times the potential gradient. Small potentiometers may be inserted at different points around a root system and without disturbing the plant the soil-moisture conditions can be carefully followed. If the conductivity and capillary potential for the soil have been investigated, then the direction and velocity of the water movement can be approximated, if not accurately determined. Potentiometers, like the one illustrated in Figure 3, can be used to measure ψ only when the tension in the liquid is between 0 and 1 atmosphere. It seems likely, though, that the optimum soil-moisture conditions for most of the agricultural plants lie well within this range.

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[13] The above discussion assumes the position of the root to be fixed. It is probable that the ability of the plant root to extend itself is an important factor in determining the "availability" of water in relatively dry soils.

[14] When there are abnormally large amounts of soluble material in the soil water, or for dryer soils, where the water is in such thin films that it ceases to have the properties of a liquid, then it would be advisable to use a function such as the free energy or thermodynamic potential. This function may be thought of as a sort of total potential which can be used in any kind of system and which would take account of the energy relations due to all of the factors such as pressure, gravity, osmosis, changes of state, chemical reactions, etc.

The "old problem," as expressed by Livingston and Hawkins (*15*), that the commonly used methods of designating or describing soil moisture condition are "not ideal for ecological or agricultural inquires," is frequently expressed in the literature. Because of the difference in the security with which water is held by a fine and a coarse soil it is obvious that expressing the water content as a percentage of the dry weight of soil is not satisfactory. Expressing the moisture content of soil as a fraction of the moisture-holding capacity gives a better idea as to the availability of the moisture to the plant, but this method has the disadvantage that for a given soil the moisture-holding capacity will depend on such things as the temperature, the state of packing of the soil, and the length of time which is allowed for the soil to drain or to reach equilibrium. Also, when it is desired to maintain constant moisture conditions for potted plants over a period of time it is necessary to resort to the rather uncertain procedure of adding water to keep the pots up to the desired weight.

Because of the significance of the capillary potential concerning the "security" with which the water is held by the soil and also because of the ease with which the potential can be measured and controlled for experimental purposes, this function seems to have advantages over any of the magnitudes now used for designating or controlling soil-moisture condition as a factor in plant environment.

MEASUREMENT OF ψ IN DRY SOILS

Under ordinary conditions, such as in pipes, glass tubes, etc., it is difficult to subject water to very great tensile stress. If the lower end of a tall vertical cylinder with a carefully fitted piston were submerged in water and the air removed from beneath the piston we could, by raising the piston, make the water stand in the cylinder to a height of 28 to 32 feet, depending on the barometric pressure. If we attempt to increase this height by further raising the piston the water simply breaks into the vapor phase because the liquid will not stand any further decrease in pressure or increase in tension. There is evidence, though, that if proper conditions are obtained the tension may be increased for beyond this value. Some workers believe it is possible for water to support tensile stresses as large as 10,000 atmospheres. The conditions in a dry soil are such that the water is under extremely high tension. When the tension is greater than 1 atmosphere, then the ordinary potentiometers can not be used for measuring ψ.

Since the vapor pressure of the soil water depends on the surface curvature of the soil solution, the vapor pressure of the soil can be measured and the corresponding capillary potential can be calculated if the vapor pressure of the soil solution at zero curvature is known.[15] Calculations based on vapor-pressure data and the wilting coefficient given by Thomas (*18*) indicate that wheat plants can grow and absorb soil moisture which is under tensions as high as 26.4 atmospheres before wilting occurs. This is the moisture condition that would have existed if the soil had been at equilibrium with a water table 896 feet lower.[16]

The measurement of ψ in comparatively dry soils might also be accomplished with apparatus having membranes by means of which the tremendous forces of osmotic solutions could be utilized. Some experimental work has been done in this laboratory toward the perfection of osmotic apparatus similar in form to the cells in Figure 5, and in which a copper ferrocyanide membrane was supported in a thin clay plate. However, as yet, no satisfactory cells have been produced.

POSSIBLE IMPROVEMENTS IN APPARATUS

There is need for improvement and perfection of apparatus for use in connection with capillary potential measurement and control. More rugged and accurate potentiometers would be desirable for the purposes of field work. Pressure gauges could be used instead of manometers. The potentiometer should be provided with a sensitive means of detecting any motion of water from porous cup to the soil and should be arranged so that the cup water pressure can be reduced by hand until the outward flow ceases. Also, for certain kinds of work, the porous clay surface could be mounted as a section in the side of a metal pipe so that the instrument could be inserted some distance in soil without seriously disturbing the plant roots or soil structure. There is need for a recording potentiometer which can make continuous records. Capillary potential records taken over a period of years would furnish interesting information about the seasonal and yearly variation in the moisture condition of lower soil layers.

There is need also for improvement and commercial development of porous clay soil pots which will give satisfactory soil-moisture control for plant experimental work and possibly even for general use. Figure 7 shows a design that has been made up in this laboratory. A is a porous clay cup, shaped like an ordinary flower pot. It is

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[15] For a system such as that shown in Figure 2 it is evident that at each height above the water table there is a definite value for the vapor pressure as well as for the capillary potential. If there were a uniform concentration of soil salts dissolved in the water throughout the system, the vapor pressure everywhere would be reduced but the capillary potential gradient in the soil water and the pressure gradient in the column of vapor would still remain. In a column of water vapor the pressure, p, at any point, is given by the exponential $p=p_o\,e-\left(\frac{\rho_{vo}gh}{p_o}\right)$, where h is the distance of the point above the level where the vapor pressure and density are, respectively, p_o and ρ_{vo}. If the vapor pressure of the water-saturated soil (zero hydrostatic pressure in the soil solution) is known, then the capillary potential in the soil at any other moisture content may be calculated from the vapor pressure of the soil by using the above formula.

Thomas found that wheat plants wilted when the vapor pressure, p, in Greenville soil was 2.330 cm. of mercury. This is 0.046 cm. less than the vapor pressure of the saturated soil at the same temperature. Thus, taking p_o as 2.376 cm. of mercury and ρ_{vo} as 23.11×10^{-6} gm. per c. c., we can calculate h from the above formula. Solving for h, we have

$$h=-\frac{p_o}{\rho_{vo}g}\,ln\,\frac{p}{p_o}=\frac{2.376\times13.6\times980\times2.3\times.0085}{23.11\times10^{-6}\times980}=27{,}336\text{ cm}$$

This is numerically equal to the capillary potential (expressed in gram centimeters per gram) because the soil would have to be at this height in a soil column to be at equilibrium with the free flat surface of the soil solution.

[16] To avoid misunderstanding, it should be emphasized that even though wheat plants may be able to use soil water under tensions as high as 26.4 atmospheres, it is quite certain that water from an 896-foot water table would be "unavailable" for plant use because the rate of water movement would be too slow to supply the needs of the plant.

sealed into the can, B, by means of plaster of Paris and soft wax. A tube is fitted into the can at C and is connected to a pipe that reaches down to a free water surface. When the can and tube are filled with water and a stopper is inserted at D the capillary potential of soil in the pot will be determined by the height, *h*. This is essentially the same principle as that used by Livingston for his auto-irrigators (*14*). The type of apparatus here described has the advantage that the porous water-supplying surface is relatively larger and leaves the inside of the pot free to be occupied by the plant roots and soil. The porous clay material described in this paper was molded and fired in the laboratory. It is likely that with improved methods it would be possible to make the capillary cell unit A and B in Figure 7 out of one piece of fired clay, thus eliminating the sealed joint.

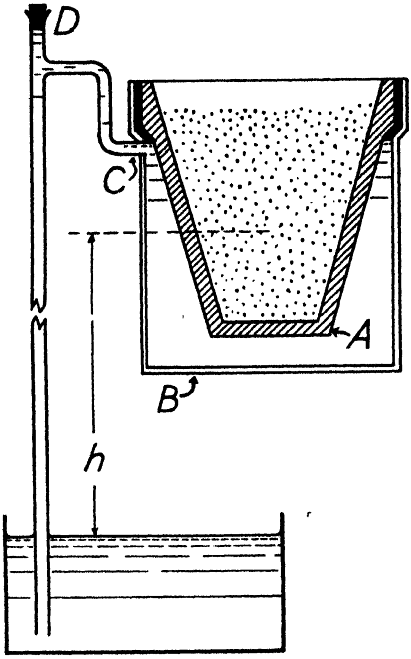

FIG. 7.—Apparatus used for controlling the capillary potential for potted plants. See text for complete explanation

There is need for the perfection of osmotic cells or other forms of apparatus for the measurement of ψ in dry soil. It is possible to extend considerably the range of usefulness of the apparatus shown in Figure 5. If the soil on the plate were covered with an air-tight case so that by supplying air pressure in this soil chamber the pressure differences between the air in the soil and the soil solution could be greatly increased. The value of ψ would be correspondingly decreased and determinations could be made at varying pressures.

FURTHER EXPERIMENTAL DATA NEEDED

The capillary potential has as yet been only slightly used or applied in experimental work. The following are some promising fields for experimentation that should yield valuable information: (1) Detailed experimental study of the capillary transmission constant for different soils with various states of packing and moisture content; (2) more complete study of the relation existing between capillary potential and soil-moisture content, mechanical composition, state of packing, temperature, and soluble salts; (3) study of optimum growth and germination potentials for different plants and experimentally determining whether or not ψ is a more significant quantity than those now used for designating and describing soil-moisture condition as a factor in plant environment.

SUMMARY

Studies on the movement of soil moisture and its availability to plants have been largely experimental and there is need of a comprehensive, guiding theory. The energy potentials and dynamical methods which have been found so useful in studying electricity and heat may be equally well employed in soil-moisture investigations.

A discussion is given on the nature and use of potential functions, and the electrostatic, gravitational, pressure, capillary, and total potentials are defined.

It is shown that, when the proper units are used, the value of the capillary potential in soil which is in moisture equilibrium with water through a porous clay wall is numerically equal to the difference in pressure in the water and atmospheric pressure. The capillary potential is therefore a measure of the pressure in the soil solution.

The factors determining the capillary potential in a moist soil are discussed and experimental data are given which show the relation between this function and the percentage of moisture for four different soils.

The flow of moisture through soil can be expressed as simply the capillary conductivity times the potential gradient, i. e., $v = -K \text{ grad } \Phi$. (This is analogous to Ohm's law for electricity and Fourier's law for heat.)

Application of the potential theory to soil-moisture movement is made for the following cases: (1) Flow of moisture downward through soil after rainfall or irrigation, (2) flow of moisture upward from a saturated water table, and (3) movement of moisture in a horizontal direction.

The availability of soil water to plants involves two factors, viz, the "security" with which the water is held by the soil and the readiness with which moisture flows in to replace that which has been used by the plant. The capillary potential is a direct measure of this "security" factor. The rate of moisture flow toward the roots is quantitatively expressed in terms of the transmission constant and potential gradient by the above equation.

A new form of porous clay apparatus is described which can be used in controlling soil water for potted plants.

The capillary potential offers a means of quantitatively expressing soil-moisture phenomena and has advantages over the commonly used methods of designating soil-moisture condition as a factor in plant environment.

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A STUDY OF THE EFFECT OF SURGICAL SHOCK ON INSECTS [1]

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SURGICAL SHOCK IN MAN AND HIGHER ANIMALS

There is a form of shock in man that sometimes follows immediately after a wound is received. This form, called primary shock by Cowell,[3] resembles fainting and is caused by nervousness. It is not the direct result of the injury itself and usually lasts but a short time.

Surgical or traumatic shock is more deep seated and results from injury to the tissues. The injured individual grows pale, his skin becomes cold and often wet with perspiration, his pulse is rapid but very feeble, his breathing shallow, and his blood pressure low. These symptoms arise more slowly and last longer than in primary shock. They follow extensive burns, injury to the intestines, muscles, bones, testes, or other tissues. They also follow prolonged etherization.

The symptoms are produced, it is suggested, by the secretion into the blood stream of substances like histamine at the time of injury. Dilation of the capillaries follows and a flow of plasma takes place from the blood through the walls of the capillaries to the tissue spaces. This decrease in blood volume results in reduced blood pressure and increased heartbeat, accompanied by a reduction in the vital functions.

A POSSIBLE COUNTERPART IN INSECTS TO SURGICAL SHOCK IN HIGHER ANIMALS

In a series of experiments conducted at this laboratory there has been found in insects a phenomenon which possibly may be interpreted as associated with traumatic shock. One of the effects is evident immediately after injury is received, but obviously the general symptoms must differ from those produced by shock in mammals.

The practical point of interest in shock of insects is, of course, the possibility that it may affect the insect's functions and cause distortion of physiological measurements, for severe injury to insects does result from some physiological tests. A knowledge of, say, the hydrogen-ion concentration of the blood of insects under various conditions is certainly of value, but in making the determinations it is necessary to puncture the insect and to express some of its lymph, and it may otherwise be mutilated. Moreover, in the determination of internal temperature the insect is pierced with the thermocouple point, for

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rectal readings are of doubtful accuracy in entomology, while there is always the danger of puncturing the wall of the rectum and causing local shock. In these and other instances the tissues are injured, and what appears to be traumatic shock is produced.

EXPERIMENTAL DATA ON SHOCK OF INSECTS

Several species of insects, especially the pupae of the Polyphemus moth (*Telea polyphemus* Cram.), were used in the tests. Shock was administered by piercing some individuals with a needle. The body contents of others were expressed. All the tests produced an effect of such a nature that a marked disturbance of some kind was indicated. An immediate change occurred in the water relations of the tissues, due to the release of a large percentage of the water held by the cell colloids, as shown in Table 1. The technic used in making determinations of "bound" water has already been described by the writer [4] and will not be repeated here.

TABLE 1.—*Effect of shock on the quantity of bound water present in pupae of Telea polyphemus*

Percentage of bound water in—		
Normal individuals	Pierced individuals	Liquid expressed from body contents
27.5	19.1	9.6
30.5	20.4	11.2
21.3	17.4	5.3
22.1	21.2	8.8
20.8	17.6	7.6
22.3	16.1	5.2
Average...... 24.1	18.6	7.9

In normal individuals an average of 24.1 per cent of the total water, as shown in Table 1, was in the bound condition. Piercing the tissues of others caused an immediate drop to 18.6 per cent, or a decrease of about 23 per cent of bound water. Expressing the body contents of other individuals caused a drop to 7.9 per cent, or the release of a still greater proportion, namely, of 67 per cent. The pupae used in these tests weighed on an average 4 gm. each, and of this weight about 71 per cent was total water. It can thus be seen that a relatively large change in the water relations takes place when shock occurs.

Fortunately, it has been possible to confirm these determinations by other data. The liberation of a large percentage of water at the time of shock would be expected to dilute the lymph and consequently to raise its freezing point. Therefore, a series of experiments was conducted to find the freezing point of lymph from normal and pierced individuals and also that of expressed lymph. The results are given in Table 2, where a rise in the freezing point is shown from −6.9° C. in normal individuals to as high as −2.1° in expressed lymph.

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TABLE 2.—*Effect of shock on the undercooling and freezing points of Telea polyphemus*

[Temperatures given in degrees centigrade]

Insects in normal condition		Insects pierced with needle		Insects pierced with thermojunction, which remained in body during determination		Expressed tissues and lymph	
Undercooling point	Freezing point	Under-cooling point	Freezing point	Under-cooling point	Freezing point	Under-cooling point	Freezing point
−15.2	−6.8	−6.7	−3.8	−7.3	−2.7	−5.8	−2.0
−15.2	−7.5	−5.2	−3.7	−6.8	−2.6	−5.1	−2.2
−15.4	−6.2	−7.5	−3.9	−5.6	−2.7	−4.7	−2.3
−15.6	−7.0	−7.6	−3.9	−6.3	−2.3	−4.8	−2.0
−15.2	−7.0	−6.5	−3.9	−6.8	−2.6	−4.8	−2.1
−15.4	−6.8	−7.1	−3.7	−6.2	−2.5	−5.4	−2.1
Average......−15.3	−6.9	−6.8	−3.8	−6.5	−2.6	−5.1	−2.1

The direct correlation which is thus established between percentage of bound water and freezing-point depression is shown in Figure 1. A theory to account for the unusual rise in the undercooling point at the time of piercing is mentioned in the paper that immediately follows the present one. In the same article a description is given of a method of taking freezing-point readings without piercing.

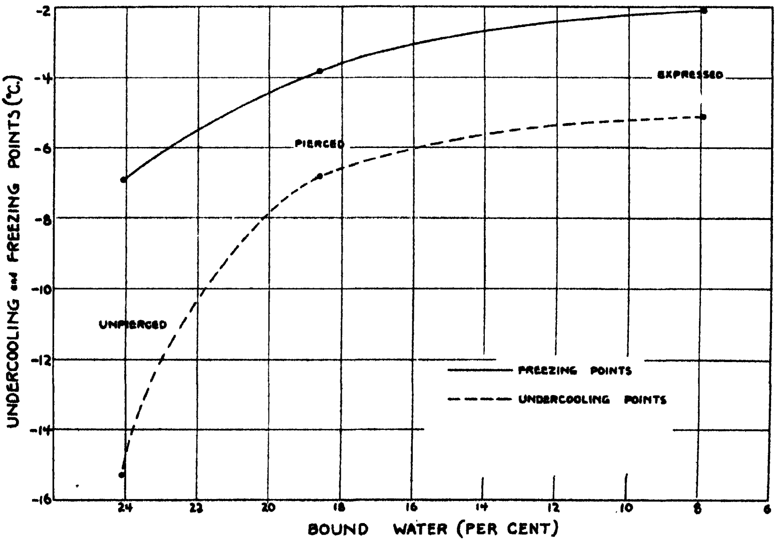

FIG. 1.—Correlation between freezing-point depression and percentage of bound water. Average of six tests

The rapidity with which shock occurs after insects are pierced is shown in Figure 2 for pupae of *Telea polyphemus*. The broken line represents the descending body temperature of a number of unpierced individuals as the cabinet temperature fell slowly during 10 days. At practically any time during the descent the effect of shock could be readily demonstrated. Piercing with the thermocouple caused an instantaneous rise in every case to the abnormal freezing point between −2.0° and −3.2° C. The difference between the normal and abnormal freezing points is further exemplified in this figure, for the unshocked pupae which were allowed to reach their normal undercooling point froze between −6.7° and −7.2°.

The rise in freezing point of over 4 degrees at the time of shock represents a large dilution of lymph and a great fall in osmotic pressure. It is difficult to account for this solely on the basis of liberation of bound water as determined. It may be that, due to shock, dissolved substances are removed from solution by adsorption.

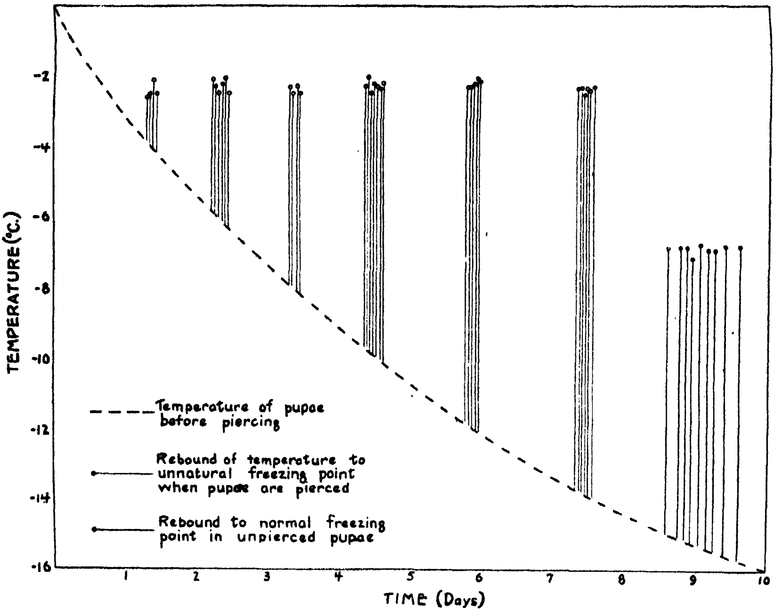

FIG. 2.—Curves showing rebound to abnormal freezing point at time of piercing and also the natural freezing point of normal specimens of *Telea polyphemus*

A DISCREPANCY IN LOW-TEMPERATURE STUDIES EXPLAINED ON THE BASIS OF SHOCK

Normal insects when placed in a low-temperature cabinet have frequently been taken considerably below the expected undercooling point without becoming frozen. Carter[5] (1925) was probably the first to point out this discrepancy, and he suggested that the higher freezing point of pierced individuals was due to injury to the tissues.

Insects evidently possess greater resistance to low temperatures than has been apparent from freezing-point determinations. There are indications of a freezing zone for the different species which exists several degrees below the expected temperatures. A series of experiments was conducted to discover the temperature difference between the normal freezing zone and the abnormal freezing points as obtained by piercing. The procedure was as follows: A large number of normal insects of different species were placed in low-temperature cabinets and their temperature was allowed to drop slowly until the predetermined or abnormal undercooling point was reached. From there downward the specimens were cooled much more slowly—about 1.5° per day. Each day several individuals were examined for the appearance of ice crystals and then discarded. It was found that every species studied could be taken below the predetermined undercooling point before crystallization began.

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[5] CARTER W. THE EFFECT OF LOW TEMPERATURES ON BRUCHUS OBTECTUS SAY, AN INSECT AFFECTING SEED. Jour. Agr. Research 31: 165–182, illus. 1925.

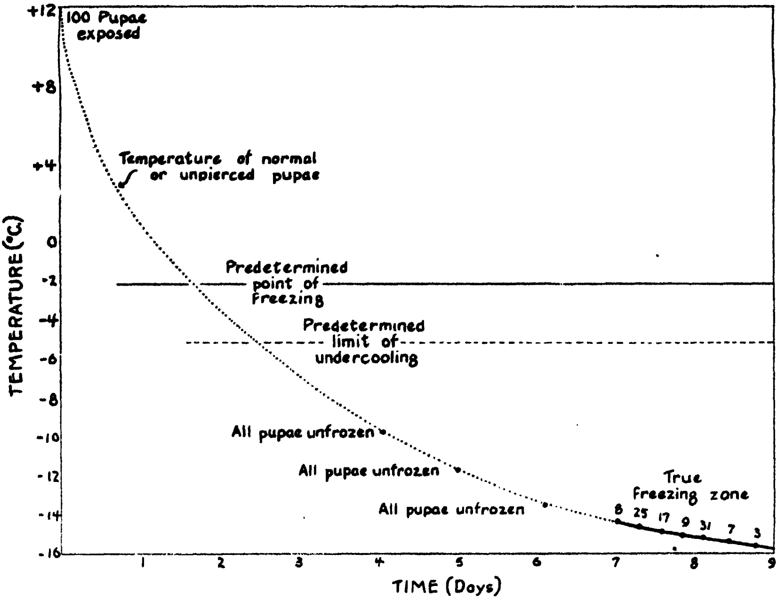

FIG. 3.—Curve showing the existence of a true freezing zone below the limit of undercooling as determined by piercing specimens of *Telea polyphemus*

The results secured for *Telea polyphemus,* which are typical of those obtained for the other species, are shown in Figure 3. One hundred normal pupae were used, and under a falling temperature they passed the abnormal undercooling point of −5.2° C. in two and a half days. The species is soft, and so freezing could be detected without dissection. The pupae were all lowered to −14.4° before freezing began, and some individuals resisted freezing down to −15.7°. This indicated a normal freezing zone between −14.4° and −15.7° for that species. Table 3 gives the results obtained for the other species in the series. In every case the normal freezing zone is several degrees below the undercooling point, and in some instances it is almost incredibly low.

TABLE 3.—*Comparison of predetermined freezing and undercooling points with normal freezing zone for all species tested*

Species tested	Average predetermined freezing point (°C.)	Average predetermined undercooling point (°C.)	Normal freezing zone (°C.)
Full-grown wireworms (*Phyllophaga* spp.)	−1.6	−3.8	−12.1 to −15.2
Colorado potato beetle (*Leptinotarsa decemlineata*)	−2.3	−4.6	−8.4 to −10.1
Pupae of Polyphemus moth (*Telea polyphemus*)	−2.5	−5.2	−14.4 to −15.7
Larvae of the willow sawfly (*Cimbex americana*)	−1.4	−3.6	−4.2 to −6.3
Pupae of the dill worm	−2.8	−4.2	−8.8 to −10.3
Pupae of the mourning-cloak butterfly (*Aglais antiopa*)	−2.2	−3.7	−7.1 to −9.9
Adults of the granary weevil (*Sitophilus granarius*)	−4.6	−8.3	−18.0 to −22.1
Black cabinet beetle (*Attagenus piceus*)	−3.2	−7.8	−15.5 to −18.6

DESIRABILITY OF USING NORMAL MATERIAL FOR PHYSIOLOGICAL TESTS

Just as piercing or mutilation obscures the true freezing point of insects, so it is possible that other tests may likewise give incorrect values. In studying the reactions of a species to the various stimuli of its environment, it is the living normal organism that should be considered. The act of taking a physiological measurement may cause disturbances sufficient to alter the true condition. The question may therefore be asked whether it is justifiable to attach much significance to values thus obtained and to interpret responses on the basis of these values.

SUMMARY

When insects are pierced, cut, or injured an effect is produced upon them that may be analogous to surgical shock in higher animals. This is most evident in the rapid change that takes place in the water relations. Since in some physiological determinations injury to the tissues is unavoidable, the data thus obtained may not accurately represent the condition in normal individuals.

DETERMINATION OF THE NATURAL UNDERCOOLING AND FREEZING POINTS IN INSECTS[1]

By WILLIAM ROBINSON

Division of Entomology, Agricultural Experiment Station, University of Minnesota

THE VALUE OF UNDERCOOLING AND FREEZING POINT DETERMINATIONS IN INSECT PHYSIOLOGY STUDIES

Although some species of hardy insects emerge normally in the spring after being frozen solid during the winter, the greater number studied at this laboratory have been found to perish if even partially frozen. There is, however, a marked difference in the susceptibility of different species to injury by ice formation within the tissues, and consequently freezing-point determinations are valuable in low-temperature studies.

Associated with the phenomenon of freezing is that temperature still lower in the scale, called the undercooling point, at which crystallization begins. With unpierced insects this has been found almost invariably to be several degrees below the freezing point. Since crystallization does not occur until the point of undercooling is reached, there is still a margin of safety for the organism; for if the temperature should cease to fall before that critical point is reached, the insect may escape freezing and death although it may have been cooled below its freezing point. In such a case it may survive if the exposure is not unduly prolonged.

The point of undercooling thus appears to be an important temperature also, since it probably represents the absolute minimum temperature for those species that are able to endure dormancy but can not survive freezing. This applies to comparatively short exposures only, for a low temperature may or may not be fatal according to the length of the exposure.

HOW THE DETERMINATIONS ARE MADE

The undercooling and freezing points of insects are well-marked temperatures and can readily be determined. The procedure is essentially the same as that used in physics and chemistry. The material is cooled to the point where the temperature abruptly ceases to fall and begins to rise. This is the undercooling point. The burst of heat during the formation of ice crystals will raise the temperature of the material to its freezing point, and it will then remain constant for a length of time depending upon the mass used or the size of the specimen.

An insect the size of, say, a Colorado potato beetle, when placed in a cabinet at about −20° C., would undergo in a few minutes an internal temperature change approximately as shown by the heavy full line in Figure 1. With the aid of a thermocouple and a potentiometer outfit any point on this line can be determined.

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[1] Received for publication Aug. 10, 1928; issued January, 1929. Published with the approval of the director as paper No. 796 of the Journal Series of the Minnesota Agricultural Experiment Station.

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From the time the specimen is placed in the cabinet until it finally reaches the cabinet temperature it continues to radiate heat from its surface, and the warmer it is the greater, obviously, will be the amount of heat given off. Therefore at the time of rebound the heat radiation is increased, but the consequent loss of heat is not sufficient to prevent its temperature from reaching the freezing point. The fact that the temperature remains constant at the freezing point for some time after the rebound, as shown in Figure 1, indicates that an equilibrium has been reached between the heat lost by radiation and that gained by crystallization. Any excess of heat generated at the time of the rebound would not be lost by radiation but would be used up in melting ice crystals already formed. A failure of the rebound to reach the freezing point would probably take the form shown by the faint dotted line in Figure 1, where the temperature would rise slowly and fall again without a pause. This, however, is a hypothetical case, for the writer has never observed it to occur even with very small insects, such as granary weevils, in which the proportion of surface to mass is large. A period of constancy at B has always been apparent.

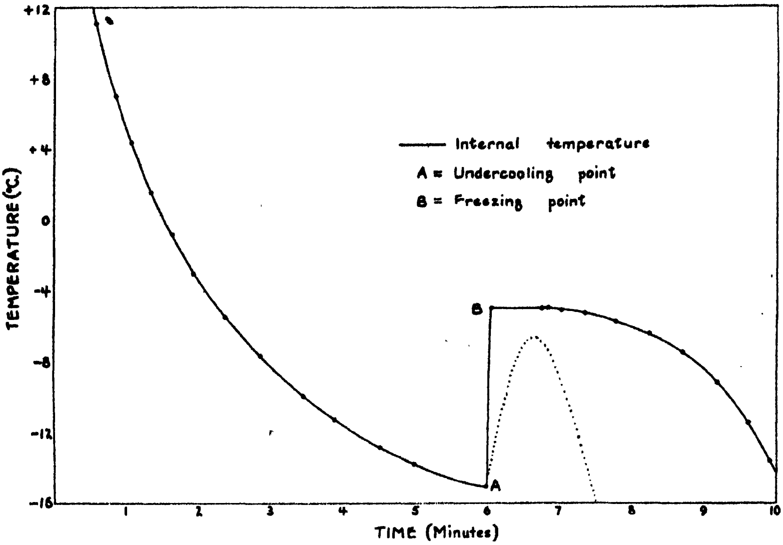

FIG. 1.—Internal temperature change of an insect the size of a Colorado potato beetle when placed in a cabinet at 20° C.

FACTORS AFFECTING THE RELIABILITY OF THE PIERCING METHOD

The most convenient method of making a determination is to impale the specimen upon the point of a thermocouple, and thus get the internal temperature reading direct. Unfortunately, however, piercing the tissues with the instrument has a physiological effect upon the organism which changes the position of the points to be determined. This effect is described by the writer in the article that immediately precedes the present one. There it is shown that when an insect is pierced a shock is produced within the organism which is accompanied by a flooding of the tissues with free water, due probably to the release of bound water held by the cell colloids. As a result, the lymph becomes diluted and the freezing point is raised.

The position of the undercooling point is still more markedly disturbed by piercing, as is shown in Figure 1 of that article. The large elevation of the undercooling point of pierced individuals is probably due to the injection of crystal nuclei at the time of piercing. The phenomenon of freezing, being a crystal-forming process, is hastened by the addition of minute particles of material, many of which adhere to the needle or thermocouple point. It will be observed in the figure just mentioned (p. 745) that although the freezing point of *Telea polyphemus* was raised 3.1° at the time of piercing, the undercooling point was raised 8.5°.

For two reasons, therefore, the piercing method, although very convenient and rapid, appears to give inaccurate results; first, because of shock, and, second, because of injection of crystal nuclei.

DETERMINATION OF INTERNAL TEMPERATURE WITHOUT PIERCING

When an insect is taken from a warm room and placed in a low-temperature cabinet standing at about −20° C., a sharp gradient of temperature is set up between the inner tissues of the insect and its surface, due to the loss of heat outward. The larger the insect the greater will be the gradient. If the temperature of the cabinet is constant the internal and surface temperatures of the insect will bear a definite relationship to each other, and if one is known the other can be estimated.

A thermojunction placed firmly against the surface of the insect will record a temperature lower than that of the surface, because the thermojunction is affected also by the temperature of the cabinet. The reading thus obtained is therefore a resultant of the two temperatures; but if the cabinet is held constant it is possible to employ the contact method to obtain the internal temperature record of the insect.

A THERMOCOUPLE FOR CONTACT DETERMINATIONS

For contact readings a slight modification of one of the junctions of the thermocouple is required. The electromotive force of the thermocouple is set up at the point where the two dissimilar metals come together, and not at the tip. Therefore it is this point of union that must always be in actual contact with the material when a determination is made. Especial emphasis must be placed upon this fact for contact determinations; otherwise inaccuracies will occur and the rebound at the time of crystallization will be obscured. Moreover, the junction should be pressed firmly against the body of the insect, but not enough to distort the shape of soft forms.

A thermojunction with holder as shown in Figure 2 has been found satisfactory. The holder consists of a piece of glass or metal tubing, A, through which the thermocouple wires are run, and which is then plugged at each end with a tightly fitting cork. The wires are run purposely between the cork and the tube, as shown in the end view of A, rather than through the cork. The thermojunction is then bent at right angles to lie against the face of the cork plug, so that the actual junction of the wires will come about the middle of the plug.

A simple device to hold the insect against the junction may be made as illustrated in Figure 2, B. It consists of a cork bored so that it will slide firmly up the tube, and to this cork a piece of tape of sufficient length is fastened with an adhesive band, such as zinc-oxide tape. The holder is then ready for use. When a determination is to be made the insect is placed in the loop of the tape and the tube is pressed down through the cork until the thermojunction comes in contact with the insect.

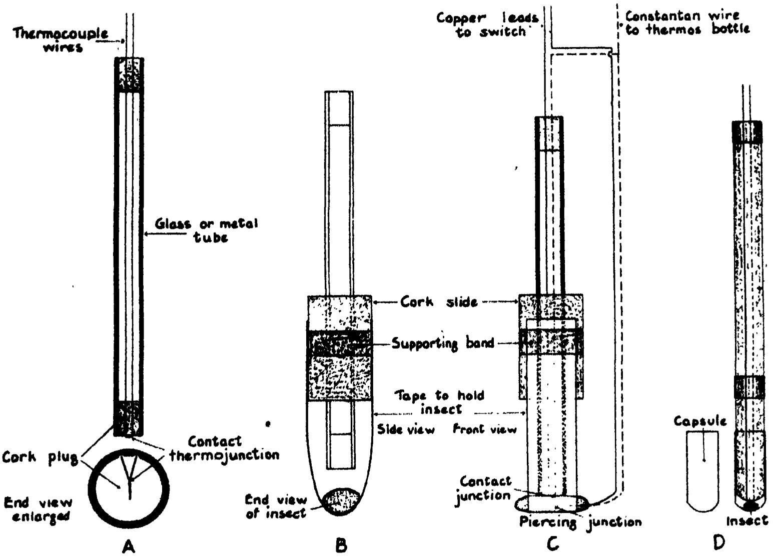

FIG. 2.—A thermojunction with insect holder for use in making contact temperature determinations. See text for detailed description

For small insects a still simpler device may be used. (Fig. 2, D.) It is made of a piece of wood rounded at one end and having a groove along two sides. The rounded end is made to fit firmly into a gelatin capsule. Capsules of this type may be readily obtained in several sizes. The thermocouple wires are run down the grooves and the junction is set at the rounded end. The insect is dropped into the capsule and the holder is pressed down until the thermocouple comes in contact with the insect.

THE CORRELATION CHART

With the contact thermocouple ready for use, it remains to determine the relation between the surface temperature as recorded by contact and the actual internal temperature of the insect. This can be done by the use of a correlation chart, as shown in Figure 3. By means of a thermocouple with combined contact and piercing junctions (fig. 2, C) both temperatures are taken close together at short intervals of time. When readings have been obtained thus for several individuals they are next plotted, as illustrated by the light dotted lines in Figure 3, the heavy full line being their average.

It is understood that the insects used in making the chart always give an inaccurate freezing point when pierced, but the actual freezing point of these individuals is of no value, since it is only the correlation between the internal and surface temperatures that is being sought. In fact, piercing in these instances has an advantage in giving a higher initial freezing point and a longer range than could otherwise be obtained.

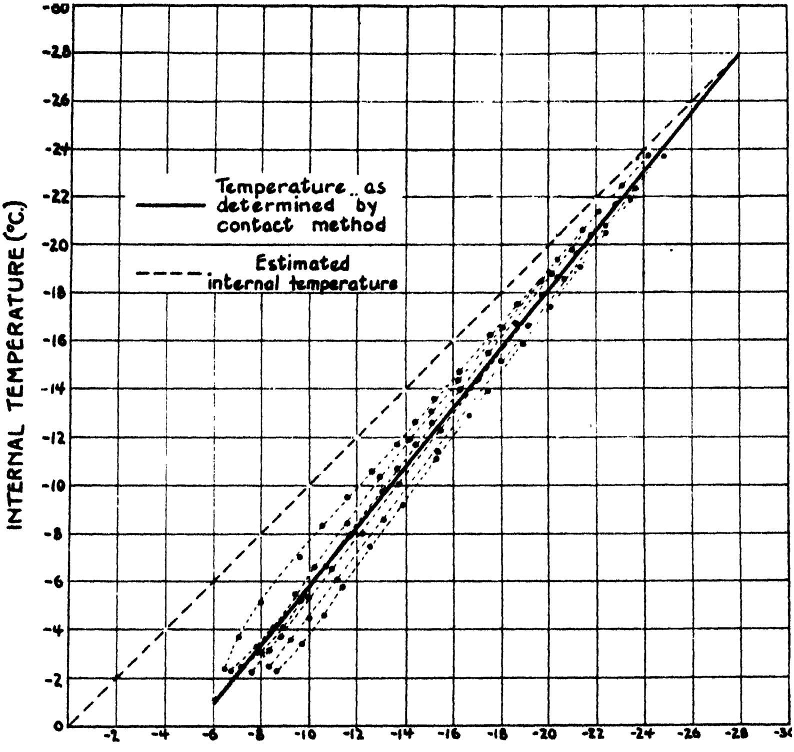

FIG. 3.—A correlation chart to be used in determining the actual internal temperature of an insect from its surface temperature as recorded by contact

The two copper leads (fig. 2, C) from the combined contact and piercing thermocouple are connected to a rotary or a 2-way switch to permit one junction to be disconnected and the other to be connected to the galvanometer at one movement. This arrangement allows readings of the internal and contact temperatures to be taken very close together, which is important. The temperature of the freezing cabinet must be held constant; and the thermojunction with holder must be placed in the cabinet and allowed to come into equilibrium with it before any readings are taken, as a varying temperature of the thermojunction holder must be avoided.

When a determination is to be made, the thermojunction holder is taken out of the cabinet, the insect is placed lengthwise in the loop, and the junction is pressed fairly firmly against the insect. The piercing junction is then inserted in the body and the holder and insect are placed in the cabinet. This should all be done as quickly as possible to prevent the temperature of the junction holder from being unduly raised. The temperature gradient between the interior and the surface is too sharp at this time to be measured accurately; that is, the two temperatures will change so quickly that if they are taken now they will not represent their correct relationship to each other. Apparently the most practicable time to begin is when the rebound to the freezing point has occurred. From there downward several readings for each insect should be taken. When readings for several individuals have thus been obtained and the data plotted to show internal temperatures on one axis and contact readings on the other, a correlation curve for the mean can be secured.

The first three or four determinations for any individual will not always follow a straight line, as seen in the faint dotted lines in Figure 3. This is probably because at the higher temperatures the insect is still soft and permits a movement of the piercing junction within its body across the sharp gradient. After freezing occurs the junction remains stationary and the line flattens out.

The chart will possibly hold only for the species for which it was made, others probably being necessary for other species. The diameter of the insect and the hardness of the surface are important limiting factors. For very small species it is difficult to make a chart. However, it has been observed that the smaller the species the less is the difference between its contact and internal temperatures. Decrease in size means a corresponding reduction in temperature gradient. The writer has found that in the case of rice weevils, which are very small hard beetles, the undercooling point, as indicated by contact, appears to be very close to the undercooling point in nature, for the weevils will not begin to freeze until approximately that temperature is reached.

It will be noticed in Figure 3 that as the exposed insects become colder their gradient decreases. The heavy full line approaches the heavy broken one, and the two meet at the temperature of the cabinet—which is exactly what would be expected. It follows, therefore, that the lower the contact reading the nearer will it be to the true internal temperature.

MAKING CONTACT DETERMINATIONS

When the correlation chart is ready it will be possible to approximate the natural undercooling and freezing points by contact, for the internal temperature can be estimated by means of the chart without piercing. For instance, an undercooling point of −18° C. by contact makes the internal temperature −15.7° for the species of insect and the type of thermojunction used by the writer. The point thus determined coincides remarkably well with that obtained by different means—that is, by placing a number of insects in a cabinet and lowering the temperature about 1° in 12 hours, and noting the point below which the insects can not go without freezing.

The thermocouple made for the combined readings may be used for these determinations also, the contact junction only being used. However, a couple with only the single contact junction will be found much more convenient. The essential features emphasized for combined readings, such as precooling of the junction holder, constancy of the cabinet temperature, and firmness of contact with the insect, apply, of course, to these determinations also. The freezing cabinet should be maintained at the temperature at which the correlation chart was made. Particular care should be taken to avoid injury to the specimens.

SUMMARY

In low-temperature studies undercooling and freezing-point determinations provide valuable data. The piercing method of making the determinations produces shock to the organism and gives inaccurate readings of temperature. A means of making determinations by contact is described and detailed instructions for the construction of a contact thermocouple are given.

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